VOLUME 35 SEPTEMBER 1957 NUMBER 5

Canadian Journal of Botany

Editor: D. L. BAILEY

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF BOTANY

(Formerly Section C, Canadian Journal of Research)

Under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research, the National Research Council issues THE CANADIAN JOURNAL OF BOTANY and five other journals devoted to the publication, in English or French, of the results of original scientific research. Matters of general policy concerning these journals are the responsibility of a joint Editorial Board consisting of: members representing the National Research Council of Canada; the Editors of the Journals; and members representing the Royal Society of Canada and four other scientific societies.

EDITORIAL BOARD

Representatives of the National Research Council

R. B. MILLER, *University of Alberta*
H. G. THODE, *McMaster University*
D. L. THOMSON, *McGill University*
W. H. WATSON (Chairman), *University of Toronto*

Editors of the Journals

D. L. BAILEY, *University of Toronto*
T. W. M. CAMERON, *Macdonald College*
H. E. DUCKWORTH, *McMaster University*
K. A. C. ELLIOTT, *Montreal Neurological Institute*
LÉO MARION, *National Research Council*
R. G. E. MURRAY, *University of Western Ontario*

Representatives of Societies

D. L. BAILEY, *University of Toronto*
Royal Society of Canada
T. W. M. CAMERON, *Macdonald College*
Royal Society of Canada
H. E. DUCKWORTH, *McMaster University*
Royal Society of Canada
Canadian Association of Physicists
K. A. C. ELLIOTT, *Montreal Neurological Institute*
Canadian Physiological Society
R. G. E. MURRAY, *University of Western Ontario*
Canadian Society of Microbiologists
H. G. THODE, *McMaster University*
Chemical Institute of Canada
T. THORVALDSON, *University of Saskatchewan*
Royal Society of Canada

Ex officio

LÉO MARION (Editor-in-Chief), *National Research Council*
F. T. ROSSER, Vice-President (Administration and Awards), *National Research Council*

Manuscripts for publication should be submitted to Dr. Léo Marion, Editor-in-Chief, Canadian Journal of Microbiology, National Research Council, Ottawa 2, Canada.

(For instructions on preparation of copy, see **Notes to Contributors** (inside back cover))

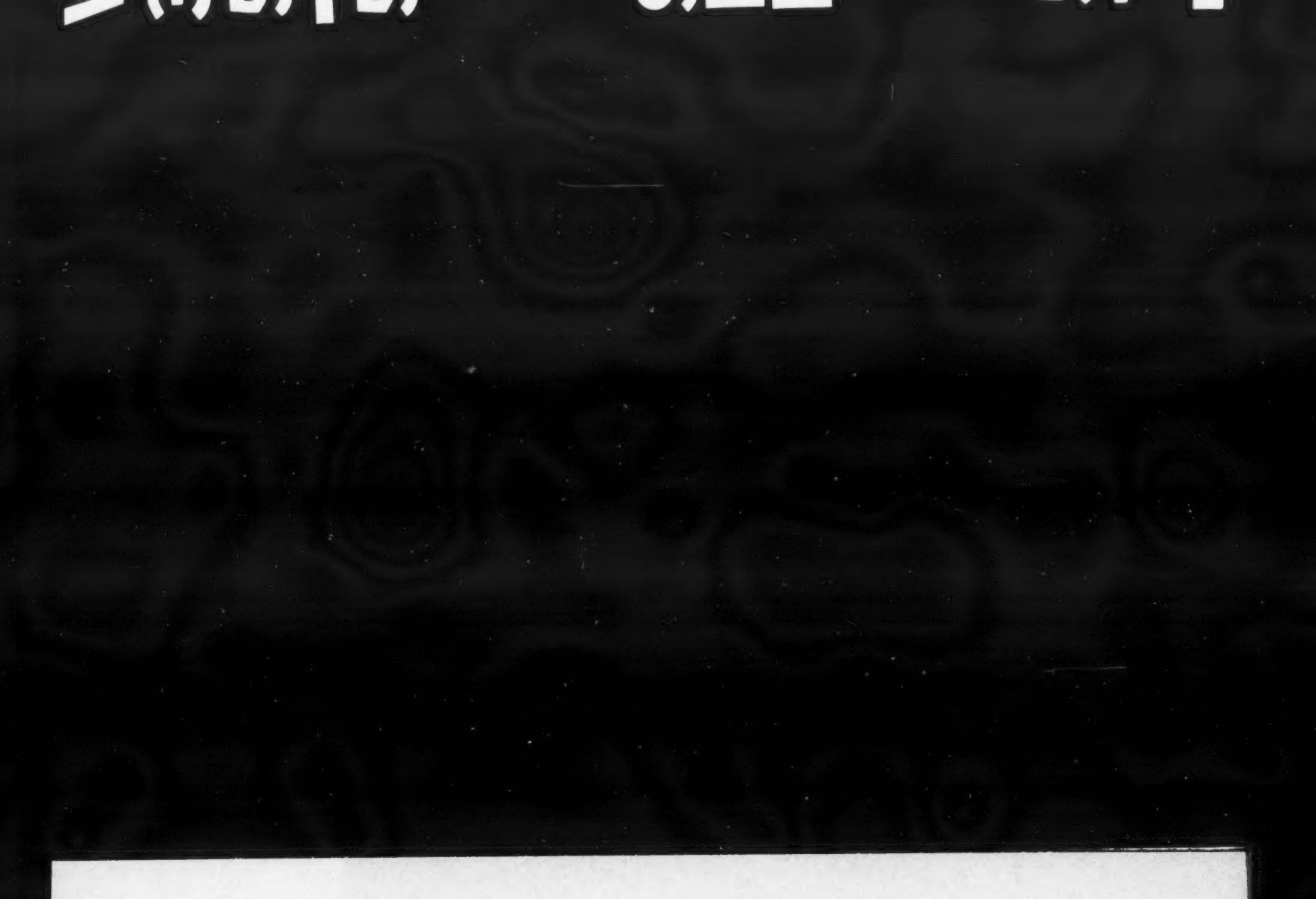
Proof, correspondence concerning proof, and orders for reprints should be sent to the Manager, Editorial Office (Research Journals), Division of Administration and Awards, National Research Council, Ottawa 2, Canada.

Subscriptions, renewals, requests for single or back numbers, and all remittances should be sent to Division of Administration and Awards, National Research Council, Ottawa 2, Canada. Remittances should be made payable to the Receiver General of Canada, credit National Research Council.

The journals published, frequency of publication, and prices are:

Canadian Journal of Biochemistry and Physiology	Monthly	$3.00 a year
Canadian Journal of Botany	Bimonthly	$4.00
Canadian Journal of Chemistry	Monthly	$5.00
Canadian Journal of Microbiology	Bimonthly	$3.00
Canadian Journal of Physics	Monthly	$4.00
Canadian Journal of Zoology	Bimonthly	$3.00

The price of single numbers of all journals is 75 cents.



Canadian Journal of Botany

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 35 SEPTEMBER, 1957 NUMBER 5

A RAPID QUANTITATIVE TEST FOR PHYTOTOXICITY AND ITS USE IN THE EXAMINATION OF A SERIES OF ALKYL-SUBSTITUTED CYCLOHEXENONES[1]

R. A. LUDWIG, ROBERT W. WHITE, AND C. H. UNWIN

Abstract

Growth inhibition in 2,4-D stimulated barley coleoptile sections can be used as a measure of phytotoxicity. Reproducible linear regression lines, showing positions and slopes characteristic of the compounds under test, were obtained. A number of alkyl-substituted cyclohexenones (5-alkyl-4-carbethoxy-3-methyl-2-cyclohexen-1-ones and 5-alkyl-3-methyl-2-cyclohexen-1-ones) were examined and found to follow the pattern of behavior anticipated for such a homologous series. In these series phytotoxicity was of a much higher order than fungitoxicity.

Introduction

A comparative study of fungitoxicity and phytotoxicity in a homologous series of N-*n*-alkylethylenethioureas has recently been reported from this laboratory (5). It was shown in this work that apparent phytotoxicity, as manifest in spraying and similar experiments, differed from true phytotoxicity as measured at a semicellular level. Tribe's tissue section technique (6) was useful in the evaluation of true phytotoxicity but the results could not be expressed quantitatively. This paper reports on a barley coleoptile growth test for phytotoxicity and its use in a comparative study in two series of alkyl substituted cyclohexenones.

The Test for Phytotoxicity

Avena coleoptile sections are utilized in a standard bio-assay procedure for plant growth regulators. Details of this technique have been published (1, 7) and form the basis for the test described here. The procedure consists essentially of measuring the growth inhibition, induced by a dosage series of a toxicant, in barley coleoptile sections stimulated with an optimal dose of plant growth regulator. Barley coleoptiles were used in preference to oat coleoptiles since they developed more uniformly and vigorously under the growing conditions employed and the authors were interested in the relationship of toxins to barley growth. Variations in growing conditions were introduced as a matter of convenience rather than of established superiority.

[1]Manuscript received April 26, 1957.
Contribution Number 105, Science Service Laboratory, Canada Department of Agriculture, University Sub Post Office, London, Ontario.

[The previous number of Can. J. Botany (**35**, 425–603) was issued July 30, 1957.]

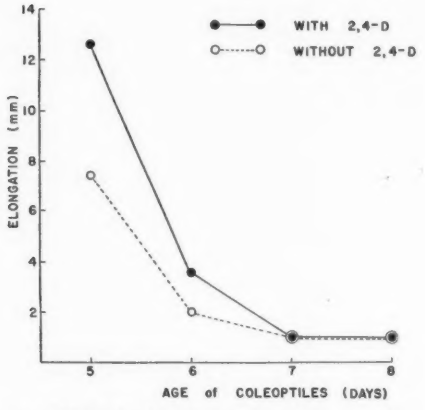


FIG. 1. Effect of coleoptile age on section growth.

Seeds of Montcalm barley were soaked in water for an hour and then approximately 1000 seeds planted at a depth of $\frac{1}{2}$ in. in vermiculite contained in a 4×6 in. flat. The flats were watered from the bottom and placed under conditions of high humidity in a darkened cabinet maintained at 65° F. in a constant temperature room. All subsequent treatments of the coleoptiles and sections were done either in darkness or under red light. Seeds were planted at noon and the coleoptiles utilized on the morning of the 5th day following. The timing was found to be critical since, as illustrated in Fig. 1, the sections rapidly lost their ability to respond to growth stimulus with age. Sections exactly 10 mm. in length were cut from the coleoptiles at a distance of 5 mm. below the apex by pressing the coleoptiles on spaced razor blades with a cork and then were transferred directly to solutions of the test chemicals in a buffer–sucrose–2,4-D medium. In practice a logarithmic dilution series of the test chemical was prepared in 3-ml. quantities of water in test tubes at twice the concentration finally desired and an equal volume of a buffer medium, then added to each (buffer at pH 6.2 ($M/12$ KH_2PO_4, four parts; $M/12$ Na_2HPO_4, one part), two parts; sucrose (20%), two parts; 2,4-D(20 p.p.m.), one part).

The resulting 6-ml. quantities were then poured into plastic boxes (43×43 ×15 mm.). Ten sections were placed in each box. The boxes, with lids on, were then incubated on a shaker at 28° C. Shaking proved to be necessary in order to achieve uniform exposure of the sections to the test chemical. Following incubation, an image of the sections was projected onto a ground glass screen at a 2× magnification with a camera lens and measured with a millimeter ruler.

2,4-D was used as a growth stimulant in preference to indoleacetic acid because of its greater stability. Initial section lengths of 10 mm. gave better results than either 20 or 5 mm. pieces. Ten sections per dish gave a reasonably

accurate reading. The mean growth of the controls from four experiments involving four lots of coleoptiles was 12.3 ± 1.13 mm. Variation was considerably less with replicates from a single batch of coleoptiles.

In calculating the results, the growth inhibition of the control was taken as 0% and the starting length as 100%. The inhibitions in the treatments were then expressed as percentages of the difference. This may introduce a slight error since the sections, as can be seen from Fig. 1, showed a non-growth increase of approximately 1 mm. The percentages of inhibition were plotted against dosage on a log–logit grid.

Dosage Response Curves for a Diverse Group of Toxicants

Maleic hydrazide, copper sulphate, 2,4-D, and 2,4-dinitrophenol were selected as a suitable group of toxicants with which to demonstrate the type of results that could be obtained with this procedure. The curves presented

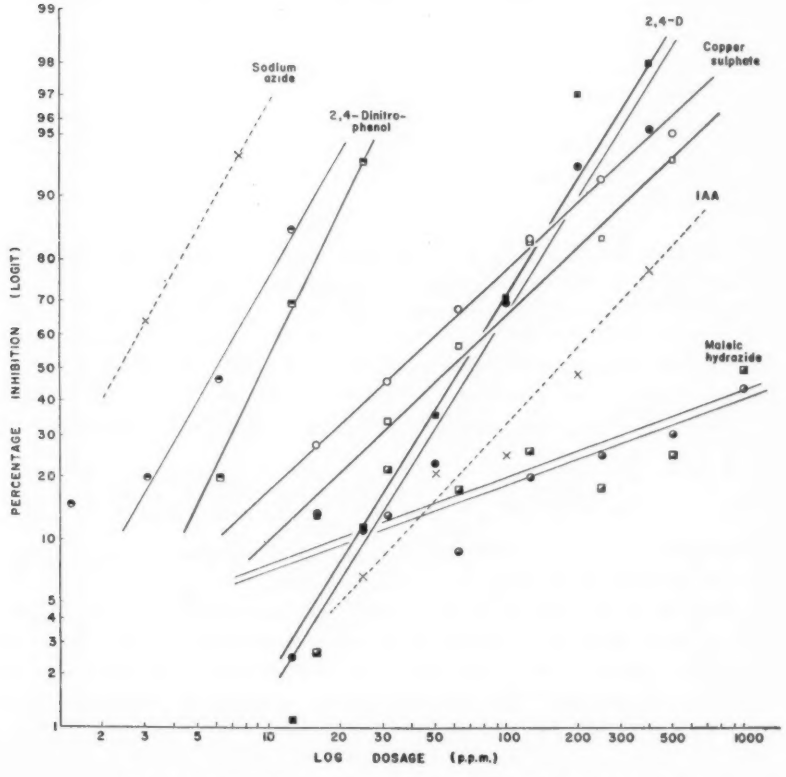


FIG. 2. Dosage response curves for a diverse series of toxicants.

in Fig. 2 were obtained in two independent experiments. The data were examined according to the method of Wilcoxon and Litchfield (8) and the resulting ED_{50} and slope values are given in Table I. All lines are a good fit and, with the exception of 2,4-D and 2,4-dinitrophenol, show significantly different slopes. Curves for sodium azide and indoleacetic acid from separate experiments have been added to Fig. 2 for purposes of comparison. It is interesting to note that the sodium azide curve falls near that of 2,4-dinitrophenol while that of IAA falls near the 2,4-D curve. The latter curves were divergent, probably because of the instability of the IAA.

TABLE I

ED_{50} AND SLOPE DATA FOR A SERIES OF TOXICANTS

Compound	ED_{50} (p.p.m.)		Slope*	
	Expt. I	Expt. II	Expt. I	Expt. II
Maleic hydrazide	1500	1500	20	20
2,4-D	71	63	2.17	2.24
Copper sulphate	35	57	3.60	3.48
2,4-Dinitrophenol	6.0	10.2	2.11	1.83

*$\frac{1}{2}\left(\frac{ED_{84}}{ED_{50}} + \frac{ED_{50}}{ED_{18}}\right)$ read from logit scale.

Reid (4) recently compared the inhibiting effects of sodium salicylate and 2,4-dinitrophenol on wheat coleoptile section growth. His growth data, when transformed to percentage growth inhibition, gave linear regression lines on a log–logit grid with Chi^2 values of 0.78 and 1.31 for the 2,4-dinitrophenol and salicylate respectively (P 0.05 = 11.1 in each case). The ED_{50} value for 2,4-dinitrophenol was 18 p.p.m. in contrast to 250 p.p.m. for the sodium salicylate. The lines were parallel with slopes of 2.74 and 2.78 thus confirming Reid's original conclusions.

Phytotoxicity in N-*n*-Alkylethylenthioureas

A detailed study of a homologous series of N-*n*-alkyl ethylenethioureas was reported by Ross and Ludwig (5). The cutoff in both fungitoxicity and true phytotoxicity was found to occur at the octyl homologue. The inhibiting effect of three members of the series on coleoptile growth was determined in the present study. The ED_{50} and slope data obtained are presented in Table II. The corresponding data on toxicity to spores of *Alternaria solani* taken from Ross and Ludwig (5) were included for purposes of comparison. The lines derived from the coleoptile data showed a significantly good fit and were parallel.

TABLE II

ED_{50} AND SLOPE DATA FROM DOSAGE RESPONSE CURVES OF N-*n*-ALKYL DERIVATIVES OF ETHYLENETHIOUREA ON INHIBITION OF SPORE GERMINATION IN *Alternaria* AND COLEOPTILE GROWTH IN BARLEY

Homologue	Water solubility*†	Spores†		Coleoptiles	
		ED_{50}*	Slope	ED_{50}*	Slope
N-*n*-Butyl	22493	8544	1.07	7607	4.17
N-*n*-Hexyl	6505	1210	1.22	699	4.39
N-*n*-Octyl	276	430	1.13	164	5.67

*M/liter $\times 10^6$.
†After Ross and Ludwig (5).

The plot presented in Fig. 3 shows the cutoff to be at the octyl homologue and is thus in accord with the earlier published work.

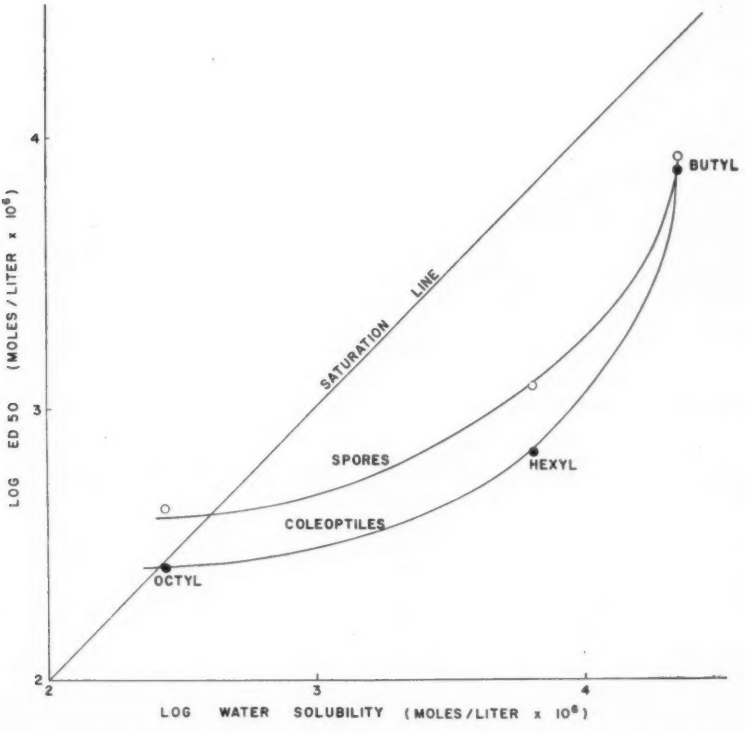
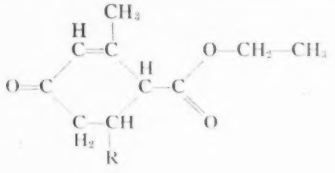


FIG. 3. ED_{50} values for the inhibition of spore germination and coleoptile growth vs. water solubility for N-*n*-alkylethylenethioureas.

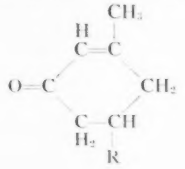
A Comparison of Phytotoxicity and Fungitoxicity in a Series of Alkyl Substituted Cyclohexenones (5-Alkyl-4-carbethoxy-3-methyl-2-cyclohexen-1-ones)

A series of alkyl substituted cyclohexenones having the general formula (I) were prepared by reacting ethyl acetoacetate with the appropriate aldehyde to give the ethyl esters according to the reaction described by Horning, Denekas, and Field (2). The corresponding saponified and decarboxylated analogues



(I)

with the general formula (II) were also prepared and examined.



(II)

Both series possess characteristic ultraviolet absorption spectra, the E_{1m} being 1209.8 at 238 mμ. Water solubilities were determined by shaking an excess of the chemical in distilled water at room temperature until optical densities at this wavelength were constant.

TABLE III

TOXICITY DATA FOR ALKYL SUBSTITUTED CYCLOHEXENONES

Series	Alkyl side chain	Solubility (moles/liter×10⁶)	Coleoptiles		Spores
			Slope	ED_{50} (moles/liter×10⁶)	ED_{50} (approx.) (moles/liter×10⁶)
Esters	H	1,401,000	3.88	3,571	> 5,000
	CH_3	857,000	3.76	4,336	> 5,000
	$—CH_2—CH_3$	228,000	4.00	1,286	> 4,000
	$(CH_2)_2—CH_3$	79,400	5.45	781	2,000–4,000
	$(CH_2)_3—CH_3$	24,800	5.86	315	1,000
	$(CH_2)_4—CH_3$	5,280	2.49	137	1,000
	$(CH_2)_5—CH_3$	1,400	3.50	105	> 3,000
	$(CH_2)_6—CH_3$	554	4.92	61	> 3,000
Saponified and decarboxylated	CH_3	1,621,000	3.53	2,540	> 5,000
	$CH_2—CH_3$	580,000	5.42	1,490	> 5,000
	$(CH_2)_2—CH_3$	164,000	4.20	487	> 5,000
	$(CH_2)_3—CH_3$	44,000	5.48	241	2,500–5,000
	$(CH_2)_4—CH_3$	10,400	4.67	306	600–1,200
	$(CH_2)_5—CH_3$	2,160	5.58	211	1,000
	$(CH_2)_6—CH_3$	409	7.24	529	> 5,000

The homologues examined and their water solubilities as well as the characteristics of their coleoptile growth inhibition curves are given in Table III. Fungitoxicity was also determined using the slide germination method with spores of *Monilinia fructiola*. Dosage response curves were not obtained since activity in this regard was found to be of a very low order. Approximate ED_{50} values are, however, included in Table III for comparative purposes.

The activities of the homologues are plotted against their water solubilities in Fig. 4. An alkyl side chain longer than that of the heptyl would obviously enhance the activity of the esters. With the saponified and decarboxylated series, however, cutoff comes between the hexyl and heptyl homologues.

Activity on plants growing in the greenhouse was checked by watering barley seedlings, growing in gravel culture, with solutions or emulsions of the compounds. A single application was made at the time of emergence. The results obtained are illustrated in Fig. 5. Here maximum activity occurred

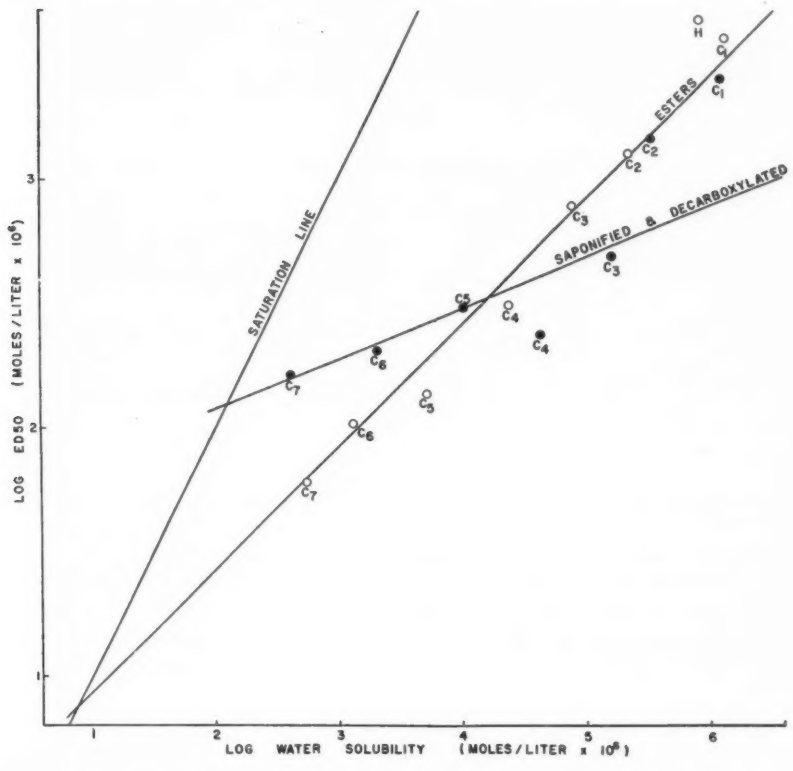


FIG. 4. ED_{50} values for the inhibition of coleoptile growth vs. water solubility for alkyl-substituted cyclohexenones.

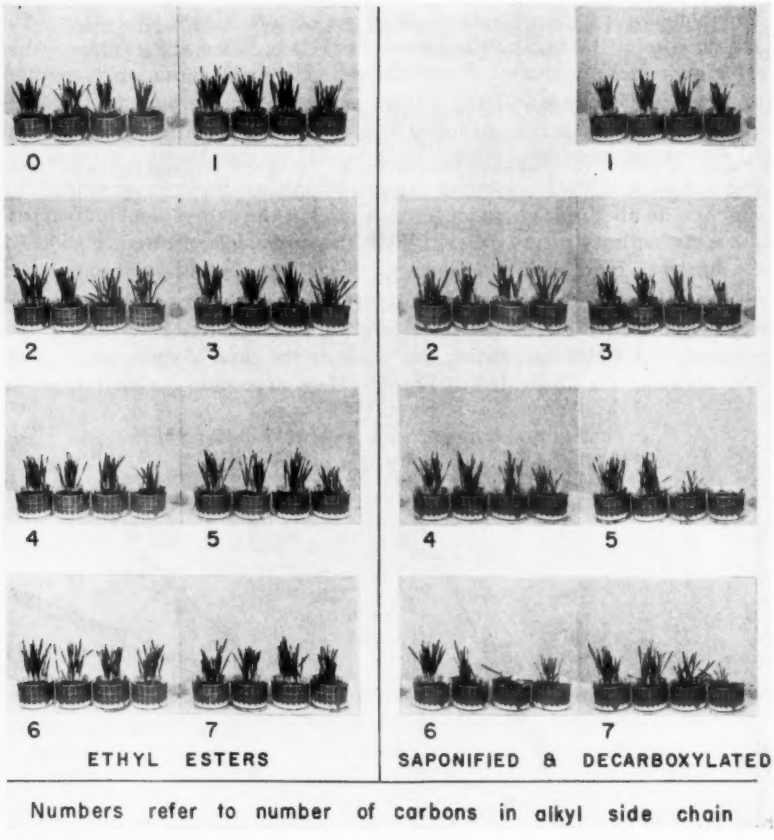


FIG. 5. Comparative toxicity of cyclohexenones to barley seedlings. Groupings from left to right are control, 100 p.p.m., 200 p.p.m., and 400 p.p.m. respectively.

in the amyl homologue of each series. This is in general agreement with the coleoptile data for the saponified and decarboxylated members but not for the esters. Further evidence of a difference in the mode of action of the two series was obtained from the type of injury symptoms produced. Treatment with the esters resulted in a burning at the leaf ends while treatment with the saponified compounds showed a white spotting of the leaf blade and, in severe cases, a white girdled area at bases of the leaves and at the gravel surface.

Discussion

Inhibition of stimulated growth in barley coleoptiles was used as a quick method of evaluating phytotoxicity. Coleoptile sections are hollow cylinders of tissue with the wall being only a few cells in thickness; hence none of the

cells are far removed from the bathing liquid. A wounding effect is introduced when sections are cut. The fact that 5-mm. pieces gave a poorer response than 10-mm. pieces was probably a reflection of the greater degree of wounding.

Derived inhibition figures for a variety of toxicants, including higher dosages of the growth regulators themselves, did not differ significantly from logit–log regression lines when tested by the Chi^2 method. Regression lines for individual compounds were found to possess slopes and positions characteristic of the compound under test. Luckwill (3) obtained straight lines with a similar type of plot in studies on the stimulating effect of plant growth regulators on tomato ovaries.

Results obtained with N-*n*-alkylethylenethioureas were in agreement with those obtained previously (5) by the use of Tribe's tissue section technique (6), the coleoptile method offering the advantage of being more quantitative and less laborious. The alkyl-substituted cyclohexenones were found to be much more phytocidal than fungicidal. Activity increased with increasing length of alkyl side chain but regression lines were parallel, indicating a similar mode of action. The ethyl esters (1) differed from their saponified and decarboxylated analogues (2) in that the cutoff in activity occurs at a much higher member of the series. Results, in this regard, agree with greenhouse findings for the latter group but not for the former.

Acknowledgments

The authors are indebted to Dr. H. Martin, Director of the Laboratory, for his interest and advice during the course of this work.

References

1. Bently, J. A. and Housley, S. Bioassay of plant growth hormones. Physiol. Plantarum, **7**, 405–419 (1954).
2. Horning, E. C., Denekas, M. O., and Field, R. E. 3,5-Dimethyl-4-carbethoxy-2-cyclohexen-1-one and 3,5-dimethyl-2-cyclohexen-1-one. Org. Syntheses Coll. Vol. III, 317–320 (1955).
3. Luckwill, L. C. A method for the quantitative estimation of growth substances based on the response of tomato ovaries to known amounts of 2-naphthoxyacetic acid. J. Hort. Sci. **24**, 19–31 (1948).
4. Reid, James. Comparison of salicylate and 2,4-dinitrophenol on the growth of wheat coleoptiles. Nature, 179 (4557), 484–485 (1957).
5. Ross, R. G. and Ludwig, R. A. A comparative study of fungitoxicity and phytotoxicity in an homologous series of N-*n*-alkylethylenethioureas. Can. J. Botany, **35**, 65–95 (1957).
6. Tribe, H. T. Studies in the physiology of parasitism. XIX On the killing of plant cells by enzymes from *Botrytis cinerea* and *Bacterium aroideae*. Ann. Botany, **19**, 351–368 (1955).
7. West, F. W. and Thimann, K. V. Phytohormones. MacMillan Co., New York. 1937.
8. Wilcoxon, F. and Litchfield, J. T. A simplified method of evaluating dose-effect experiments. American Cyanamid Co., Stamford Research Laboratories, Stamford, Connecticut.

A METHOD FOR THE STUDY OF DECAY RESISTANCE IN WOOD UNDER CONTROLLED MOISTURE CONDITIONS[1]

D. E. ETHERIDGE[2]

Abstract

A technique which employs a new method of adjusting and controlling the moisture content and employs new methods of sterilizing and inoculating the test pieces is described for studying the decay rate of wood in culture. The moisture content is expressed as a percentage of the moisture present at saturation rather than as a percentage of the dry weight; thus a uniform moisture content in test pieces of different specific gravities is obtained. Sterilization is with propylene oxide gas. Inoculations are made with small pieces of wood infected with the test fungus. The natural properties of the wood are essentially unchanged by this procedure.

Introduction

To investigate factors that might account for the different rates of decay found in fast- and slow-growing spruce trees in Alberta, laboratory experiments were carried out on the effect of variations in moisture content, specific gravity, ring frequency, and the chemical properties of wood on the amount of decay caused by *Coniophora puteana* (Schum. ex Fr.) Karst. For a statistical assessment of these factors the experimental design was to hold the moisture content constant at two uniform levels and to minimize any effect on decay which might result from inoculating, oven-drying, or sterilizing the test blocks. The standard techniques usually employed in experimental work on the decay of timber were for the most part unsatisfactory, and it was therefore necessary to modify these or devise new ones which would achieve the object of the experiment. A brief description of the techniques that were finally adopted are presented, as some of these might have general application.

Test Blocks

Samples of heartwood approximately $1\times1\times6$–7 in. were cut from fast- and slow-grown trees to obtain test-blocks of different ring frequencies and specific gravities. These were maintained at the temperature and relative humidity of the laboratory until required for the experiments. To provide adequate replication for statistical treatment of the results, nine blocks of wood measuring approximately $1\times1\times\frac{3}{4}$ in. were sawn in sequence from each sample. One end piece was selected for use in determining the moisture content of the sample. In the remaining eight pieces holes were drilled half way through one side of each block to receive a water amendment and a plug of wood inoculum as shown in Figs. 1 and 2. The nine blocks were then numbered and weighed. The oven-dry weight of each of the drilled blocks was calculated, assuming that they had the same moisture content as the selected end blocks which had been oven-dried at 105° C. for 24 hours.

[1]Manuscript received May 8, 1957.
Contribution No. 390 from the Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2]Forest Biology Laboratory, Calgary, Alberta.

The end blocks used for the moisture determinations were also used to determine the specific gravities and the ring frequencies of the samples. The specific gravity was calculated from the oven-dry weight and the volume of the wood at a moisture content above the fiber saturation point, i.e. the green volume. To bring the blocks to the green volume they were soaked in water for 24 hours, and then immersed in mercury in a graduated cylinder; the volume of the blocks was estimated to the nearest milliliter. To eliminate some of the differences in the moisture content due to differences in specific gravity, the moisture content was expressed as the percentage of the cell cavity which contained water, i.e. percentage of saturation. These determinations were calculated according to the following formula given by Brown, Panshin, and Forsaith (2), which uses the specific gravity based on the volume of wood when the moisture content is above the fiber saturation point:

$$\text{Percentage of saturation} = [(M - 28)\ SG] / (1 - 0.93\ SG),$$

where M = conventional moisture content,

and SG = the specific gravity of the wood based on the green volume assuming the fiber saturation point to be at 28% of the oven-dry weight and the specific gravity of the dry wood substance to be 1.46.

Inoculum

The best method of inoculating the test blocks was found to be by the use of disks (about 22 mm. in diameter) or plugs (about 6 mm. in diameter) of wood, as shown in Figs. 1 and 2. These were placed in 250-ml. flasks on a layer of spruce sawdust (about 3 g.), to which was added 5% of an accelerator recommended by Badcock (1). Water was added at the rate of 170% of the oven-dry weight of the sawdust. After sterilization at 15 lb./sq. in. for 30 minutes, the flasks were inoculated with plugs of agar taken from a 2- to 3-week-old plate culture of the test fungus. The pieces of wood were then incubated with the fungus for 3 months. The moisture content of the wood was kept at about 70 to 80% (oven-dry weight) by periodically removing one of the plugs of wood from the flask, determining its moisture content, and then making the necessary adjustment to the remainder.

Decay Cultures

Wide-mouthed jars 5.5 cm. × 5.5 cm. with aluminum screw-top lids were used as shown in Fig. 3. The glass jars were thoroughly washed, rinsed in distilled water, and then sterilized in an oven for 2 hours at 160° C. The wood blocks were sterilized with propylene oxide gas in the way described by Hansen and Snyder (3). The blocks were placed on a perforated tray in a large desiccator into which propylene oxide was introduced at the rate of 1 ml. per liter of the container. The blocks were left overnight, the gas was then removed with a suction pump, and the vessel flushed a number of times with filtered air. This treatment had a negligible effect on the moisture content of the blocks.

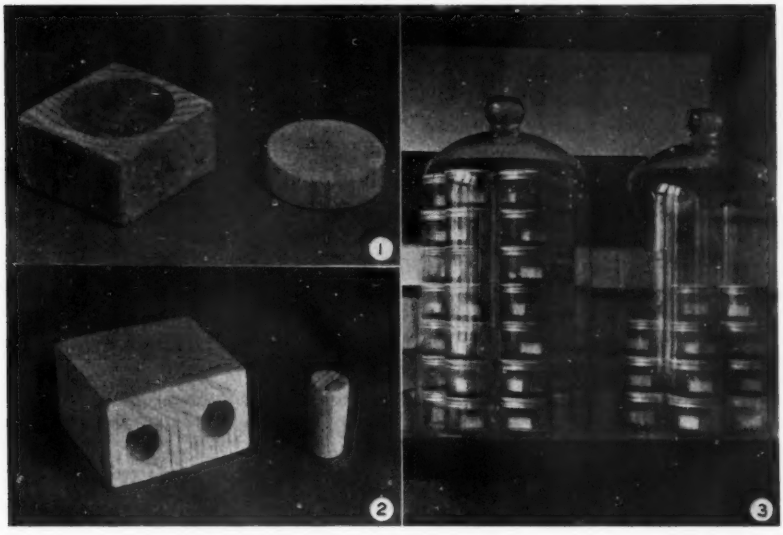


FIG. 1. Test block where the method of inoculating is with a disk of infected wood. The moisture amendment is added to the cavity.

FIG. 2. Test block where the method of inoculating is with a plug of infected wood. The moisture amendment is added to both cavities.

FIG. 3. Decay chambers showing how the test blocks are incubated in small screw-top jars in a saturated atmosphere. Water is kept in the tray under the bell jars.

Adjusting the Moisture Content of the Blocks

The treated blocks were transferred to sterile culture jars but, before the inoculum was added, the required moisture content was obtained by adding sterile distilled water from a hypodermic syringe calibrated to 0.1 ml. to the holes drilled in the sides of each block. The amount of water added took into account that which was already in the block and the specific gravity of the wood. After this initial adjustment to the moisture content was made, the blocks were inoculated with the test fungus. The lids were then loosely screwed down on the jars and they were immediately placed in a saturated atmosphere in humidity chambers as shown in Fig. 3. To maintain a constant and uniform moisture level, adjustments were made to the moisture content of the blocks during incubation. About midway through the incubation period, the blocks were removed from the jars in a room exposed for 1 hour to ultraviolet light from a germicidal lamp. After the disk of inoculum was removed from the blocks, they were weighed aseptically to the nearest 0.01 g. on a Joly spring balance, the pan of which was contained in an inoculating-hood. The water content of the blocks was then calculated; if it was above the level at which the test was being run, blocks were allowed to lose water by being kept on the laboratory bench for a few days; if it was too low, the necessary amount of water was added from a hypodermic syringe. Little

contamination followed this procedure. The decay activity was measured as the loss of weight during incubation as a percentage of the original oven-dry weight.

The chief advantages of this procedure for comparative studies of the decay of timber may be listed as follows:

1. By oven-drying only one block from each sample for moisture determinations and by surface sterilizing the blocks with a fumigant such as propylene oxide gas, tests can be carried out with wood that has not been preheated.

2. By inoculating the test blocks with pieces of infected wood, instead of following the usual practice of placing test blocks on the surface of actively growing cultures (of the test fungus) grown in nutrient media, the chances of introducing growth-promoting factors with the inoculum are reduced. Moreover, the mode of infection that frequently occurs in nature is simulated by the use of wood inoculum.

3. By expressing the moisture content as a percentage of the cell cavity occupied by water, differences in the moisture content of the wood resulting from differences in the size of the cells are eliminated. Using the conventional method of expressing moisture content, i.e. oven-dry weight basis, the proportion of the cell that is occupied by water varies with the specific gravity of the wood.

4. Finally, the procedure permits the adjustment of the moisture content of the test blocks during the period of incubation. This reduces the considerable variation that frequently occurs between the initial and final moisture content values of the test blocks.

References

1. BADCOCK, E. C. New methods for the cultivation of wood-rotting fungi. Brit. Mycol. Soc. Trans. **25**, 200–205 (1941).
2. BROWN, H. P., PANSHIN, A. J., and FORSAITH, C. C. Textbook of wood technology. Vol. II. 1st ed. McGraw-Hill Book Co., Inc., New York, N.Y. 1952.
3. HANSEN, H. W. and SNYDER, W. C. Gaseous sterilization of biological materials for use as culture media. Phytopathology, **37**, 369–371 (1947).

STUDIES ON HATCHING STIMULATION IN THE BEET NEMATODE, HETERODERA SCHACHTII SCHMIDT[1]

R. D. WINSLOW[2] AND R. A. LUDWIG

Abstract

Hatching of the beet nematode found in Ontario was stimulated by hatching factors in host plant leachates. Sugar beets produced more active root leachate under 'short' day and moderate temperatures than under 'long' day and more extreme temperatures. When beet or rape roots or germinating rape seedlings came in contact with excess water, the hatching factor was evidently liberated into the water continuously but progressively more slowly. Increasing the concentration of rape seedlings in water increased correspondingly the potency of the resulting leachate, the maximum concentration giving activity at a dilution of 1×10^6. Stimulatory leachates were produced from various parts such as tops alone, roots alone, and various portions of the root of young rape seedlings.

Sugar beet leachate was concentrated 20-to-40-fold, by vacuum distillation, without obvious loss of activity, frequently with apparent gain. Further concentration caused precipitation of active material. Attempts to extract the factor from beet and rape root leachate with charcoal, phenol, 1-butanol, or ion exchange chromatography are described. The results show points of similarity and difference amongst nematode-hatching factors.

Introduction

Baunacke (1) was probably the first to carry out laboratory investigations on the stimulation of hatching of the beet nematode (*Heterodera schachtii* Schmidt) by root leachates. He found that larvae emerged more readily from nematode cysts in water which had been in contact with the roots of host plants than from cysts in untreated water. Subsequently, most of the experimental work on the nematode-stimulating activity of root leachates has been concentrated on the golden nematode (*H. rostochiensis*) problem and methods for the mass production of potato and tomato root leachates have been published (2, 7). Work has been done on some of the factors influencing the production of active potato root leachate. Fenwick, for example, has shown that there is a close correlation between rate of root growth and potency of the leachate produced (16). The rate of breakdown of potato root leachate in soil has also been studied (9). There are, however, no published data on the influence of growth conditions, e.g. light and temperature, on leachate potency. The work described below includes experiments on this aspect of the problem and on leaching techniques. Experiments on some aspects of the production of active leachate from germinating rape seeds are also described.

There have been several publications in the past decade on the problems of isolation and identification of nematode-stimulating factors from plant leachates. Most of these have dealt with the golden nematode hatching factor, the first major work being that of Todd and his co-workers in England

[1]Manuscript received May 6, 1957.
Contribution No. 106, Science Service Laboratory, Canada Department of Agriculture, University Sub Post Office, London, Ontario.
[2]Current address: Rothamsted Experimental Station, Herpenden, Herts, England.

(2, 3, 13, 14, 17, 18). Publications followed from the United States (15), England (10, 5), and Holland (12). Preliminary work on the isolation of hatching factors of the beet, cabbage (*H. cruciferae*), and hop (*H. humuli*) cyst nematodes was carried out by Carroll (4) in England, while Wood and Serro (22) in the United States examined sugar beet root leachate by paper chromatography and drew tentative conclusions as to the possible hatching stimulants therein. In experiments described below, attempts were made to apply to the problem of beet nematode hatching factors some of the first steps in purification which had been used more or less successfully in the case of the golden nematode factor.

The Bio-assay Technique

Cysts of the beet nematode, *Heterodera schachtii* Schm., were used for bio-assay purposes. In the earlier experiments, the cysts were separated by a flask flotation technique (20), from infested soil obtained in the Blackwell district, Essex County, Ontario. Preliminary tests showed that hatching from these cysts was stimulated by leachates from the host plants sugar beet, rape, and radish, but not by leachates from the non-hosts tomato, flax, and wheat. Cysts from this source were not wholly satisfactory as assay material, however, since many of them were either empty or of low viability. In later experiments new cysts, produced on rape plants grown in beds of infested soil or in pots of gravel infested by adding hatched larvae, were used. The procedure entailed placing batches of 100 or more cysts in each of the fluids under test contained in small Petri dishes, and incubating them at 24° C. After a suitable period, usually 7 days, the hatching fluid and hatched larvae were drawn off from each dish and the larvae counted. If the hatching test was to continue, fresh hatching fluid was added to the cysts in the dish and they were returned to the incubator. Control dishes containing cysts in distilled water were included in every experiment. The potency or hatching potential of each sample of fluid tested was gauged from comparison of hatching in the sample with hatching in the controls. Cysts from different samples of infested soil differed greatly in hatchability, thus comparison between experiments is not justifiable unless hatching in the controls was comparable.

Experiments with Root Leachates from Growing Plants

Plants used in these experiments were grown in clean gravel contained in either 6-in. clay pots or small beds (2 ft.$\times$2$\frac{1}{2}$ ft.$\times$6 in. deep) and fed once or twice weekly with a simple nutrient solution (15:30:15 commercial starter). Symptoms of minor element deficiency were rare and disappeared after a single application of a complete nutrient solution. The plants were kept outdoors in warm weather and in a greenhouse in cold weather. Pots and beds were flushed through with tap water 24 hours before leaching, to wash out excess nutrient.

Leaching Techniques

A number of experiments were undertaken on methods of washing the active principle from gravel beds in which sugar beet or rape plants were growing. In one type of experiment, beds of vigorously growing beet plants were filled with distilled water. Samples of the fluid or leachate, taken after 2, 4, 8, and 24 hours in the bed and bio-assayed, gave hatches of 1.5, 2.7, 12.6, and 13.5 larvae per cyst respectively as compared to a water hatch of 0.8. The hatching power of the leachate, in other words, increased rapidly during the first 8 hours and less rapidly thereafter. These results are based on a number of experiments carried out in the summer in outdoor beds, when the first 8 hours occurred during bright daylight whereas a considerable part of the remaining 16 hours was passed in darkness. 'Circulating' the fluid (by repeatedly drawing it off and pouring it back into the bed) during the first 8 hours apparently had little effect on potency. The results as a whole indicated that leachate could be produced conveniently by flooding the beds for 8 hours and then withdrawing the fluid.

The rate of increase in potency during the first 8–10 hours was examined in other experiments. In one of these, 10 consecutive 1-hour leaches were taken from a bed of rape plants over the period 8 a.m. to 6 p.m., the fluid being drained off at the end of each hour and the bed refilled with water. Of the 10 lots of leachate thus produced, five were diluted to $\frac{1}{4}$ strength and bio-assayed. The results, Fig. 1, show that there was a progressive fall in the potency of the leachate, indicating that the hatching factor was apparently liberated into the water continuously but progressively more slowly.

Effects of 'Daylength' and Temperature on the Potency of Root Leachate Produced by Sugar Beet Plants

The effect of growing conditions on leachate potency was investigated in a series of experiments. In one of these experiments the effects of 'daylength'–temperature combinations were studied. Sugar beet seed was sown outdoors in summer in pots of a gravel–sand (2:1) mixture. When they were 7 weeks old the seedlings were thinned to 10 per pot, and six pots were assigned to each of five 'daylength'–temperature combinations, including three variations in 'daylength'—8 hours daylight through glass; the same extended to 16 hours with incandescent lighting, and unrestricted daylight (outdoors). On two occasions (2 weeks and 5 weeks after treatment began) each pot was leached by adding distilled water until 250 ml. had passed through. This was passed a further nine times through the pot. The results of bio-assays on these leachates are presented as treatment totals in Fig. 2. On both occasions and at both 'daylengths' less potent leachates were produced in the hot than in the cool greenhouse. Also, 16 hours 'daylength' was inferior to 8 hours and all four greenhouse treatments were inferior to the outdoor treatment.

In experiments carried out in winter, young sugar beet plants in pots of gravel were held at two greenhouse temperatures (50° and 65° F.) and at 'daylengths' ranging from 8 to 16 hours. The 8-hour treatment consisted

entirely of daylight, the others of daylight extended to the required number of hours with incandescent lighting. The pots were leached, in the manner described above, about 1 month after treatment began. Assay of these leachates showed that at all 'daylengths' more potent fluid was produced at 65° than at 50°, and that at both temperatures, 'short' day treatments (8–10 hours) yielded more potent fluid than did 'long day' (14–16 hours) treatments.

In another experiment carried out in winter, 8-week-old sugar beet plants in pots of gravel–sand were held at 'soil' temperatures, of 55°, 60°, 65°, 70°, and 80° F., in Wisconsin type soil temperature tanks. Leachates obtained

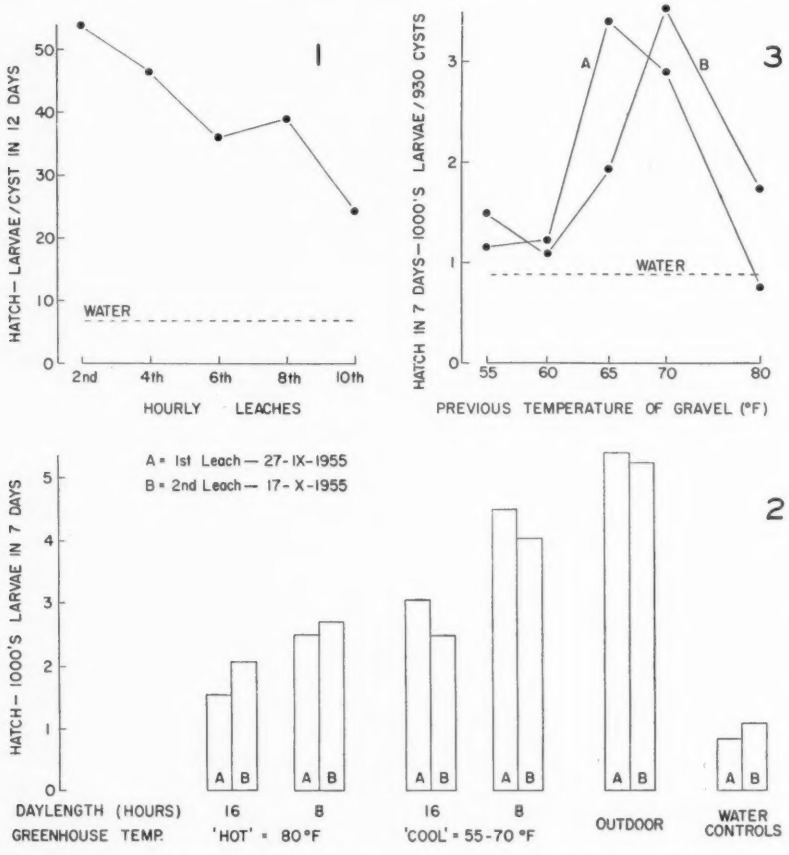


FIG. 1. Effect of repeated leaching on potency of leachate from rape plants growing in a gravel bed.

FIG. 2. Potency of root leachate from sugar beet plants growing in pots of gravel under various 'daylength'–temperature combinations.

FIG. 3. Effects of previous temperatures treatment on potency of leachate from sugar beet plants grown for 14 days (A) and 22 days (B) at 60° F.

5 weeks later gave hatches of 4.2, 6.6, 2.4, 0.5, and 0.2 larvae per cyst respectively, as compared to a water hatch of 1.2, even though there were no marked differences in the growth of the plants at the various temperatures. The 60° apparent temperature optimum was, however, obviously related to other growing conditions since, on repetition of the experiment in April under improved greenhouse conditions, a similar peaked curve was obtained with an optimum of 70° F.

Duplicate pots of sugar beet from each treatment of the preceding experiment were transferred to each of three temperature tanks held at 48°, 60°, and 72° F. These pots were subsequently leached and the leachates assayed. Leachates from the pots transferred to the 48° and 72° tanks showed no activity regardless of the previous temperature. A pronounced persistent effect of previous temperature treatments was apparent, however, in leachates from pots transferred to the 60° F. tank. This is illustrated in Fig. 3 where the results obtained from leachates taken 14 and 22 days are shown in A and B respectively.

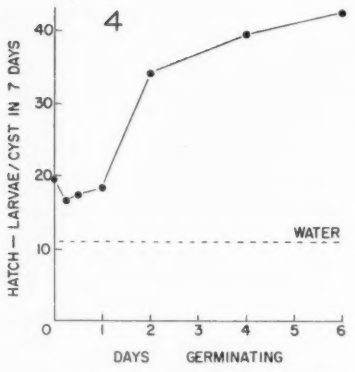
Experiments with Germinating Rape Seeds

Active leachate was produced by immersing germinated seeds of rape, radish, or sugar beet in distilled water. As germination of the sugar beet was slow and uneven, rape was used in all the experiments described below. In a number of tests, rape seeds were placed to germinate on moist filter paper, with or without pretreatment by immersion for 5 minutes in a proprietary brand of hypochlorite solution diluted with water to give a 2% available chlorine solution. Randomly chosen samples of the seeds were leached after germination periods ranging from 1 to 6 days. Bio-assay of such leachates showed that pretreatment of the seeds with chlorine had no significant effect on leachate potency. The pretreatment, however, appeared to accelerate germination somewhat and to reduce incidence of disease; consequently it was adopted as a routine measure in bulk germination of seeds.

Effect of Stage of Germination and Duration of Leach on Potency

Rape seeds were germinated, without pretreatment, on moist filter paper in Petri dishes in the dark at 72° F. Samples of leachate were taken at various intervals over the next 6 days. In each case 20 seeds chosen at random were immersed in 8 ml. water for 1 hour. Each sample was tested at three dilutions on duplicate batches of cysts. The results, presented as treatment totals in Fig. 4, were confirmed by other tests. A germination period of 2–3 days appeared desirable to produce a leachate clearly superior to water as a hatching medium. Increasing this period led to an increase in potency but also caused difficulties such as interlocking of seedling rootlets and attachment to the underlying filter paper.

In various experiments, leachates were taken from seedlings immersed in water for periods varying from 1 minute to 8 hours. Bio-assay of these leachates indicated that the potency of the leachate increased continuously



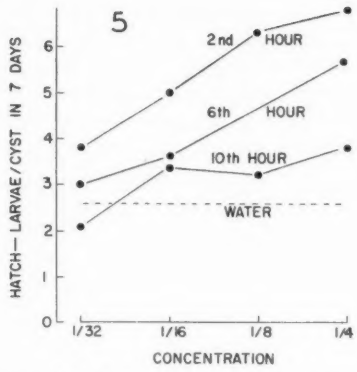


FIG. 4. Effect of germination period on potency of leachate from germinating rape seeds.

FIG. 5. Effect of duration of leach on potency of leachate from germinating rape seedlings.

but progressively more slowly. For example, leachate produced over 8 hours was less than eight times as potent as that produced in the 1st hour. This in turn was less than 60 times as potent as that produced in the 1st minute. These results were confirmed and demonstrated as follows. Ten consecutive 1-hour leaches were taken from a batch of 100 seedlings, each leach yielding 200 ml. leachate. Three of the 10 leachates thus produced were bio-assayed at a range of concentrations. The results (Fig. 5) show clearly the progressive fall in potency of the three leachates. These results are consistent with those found earlier for older plants growing in gravel.

Effect of Seedling Concentration on Leachate Potency

Leachates produced by immersing different numbers of germinating seedlings in 2 ml. of distilled water were tested on batches of nematode cysts. Hatches of 50, 62, 71, and 79 larvae per cyst were obtained from 1, 2, 4, and 8 seedlings respectively, indicating a straight-line relationship between number of larvae hatching and the logarithm of seedling concentration, a relationship very reminiscent of Fenwick's 'dilution curve' (8), where hatch was shown to vary similarly with logarithm of leachate concentration. This suggests, then, that the potency of the leachate from germinating rape seedlings is directly proportional to seedling concentration. In other experiments this was shown to be the case for seedling concentrations ranging from 1 to 64 per 5 ml. water.

The practicability of producing highly potent leachate from seeds germinated in bulk was investigated, as a possible method for the production of starting material for studies on the chemistry of nematode-hatching factors. Frequent washing and good aeration during the germination period were desirable in that these conditions resulted in uniform germination and comparative freedom from microbial contamination. An apparatus was designed in which seeds were alternately washed and aerated. In one test,

100 g. of rape seed (approximately 33,000 seeds) were washed with tap water at half-hourly intervals, the wash water being drawn off quickly each time and air drawn through the mass of seeds in the intervals between washes. The seeds germinated in 1–2 days and grew satisfactorily for another 7–10 days, eventually yielding a liter or more of very potent fluid at a single leach, taken by just covering the mass of seedlings with distilled water for 1 hour. When 10 such leaches, totalling 8 l., were taken from a single 100 g. lot of seed and combined, the pooled sample, as shown in Fig. 6, was active at a dilution of 1/1,000,000. The low over-all hatch shown here was probably due to the use of freshly-formed cysts as assay material.

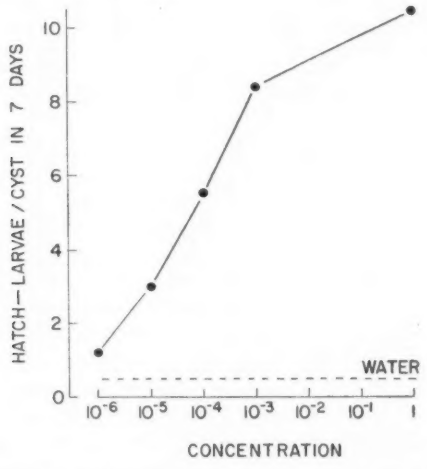


FIG. 6. Potency of leachate produced from rape seeds germinated in bulk.

The Production of Hatching Stimulant by Various Parts of Rape Seedlings

The activity of leachate from whole seedlings was compared with that produced by roots and tops. In an initial experiment, rape seeds were germinated on moist filter papers. Three days later, 20 whole seedlings or portions were placed in 10 ml. of distilled water for 1 hour. The leachates were diluted to 1/16 (i.e. to 8 ml. water per unit) for assay with batches of approximately 500 cysts. The results, given in Fig. 7A, show that very little, if any, stimulation of hatch was caused by the seed hulls, and that either excised root or top portions produced leachate as potent as that from whole intact seedlings.

In another experiment, top and root portions of intact seedlings were leached separately, using the special holder shown in Fig. 9. These leachates were compared with those from whole intact seedlings, excised roots and tops, and whole seedlings cut in two. Duplicate samples of leachate thus produced were assayed, leachates from whole seedlings being diluted twice as much

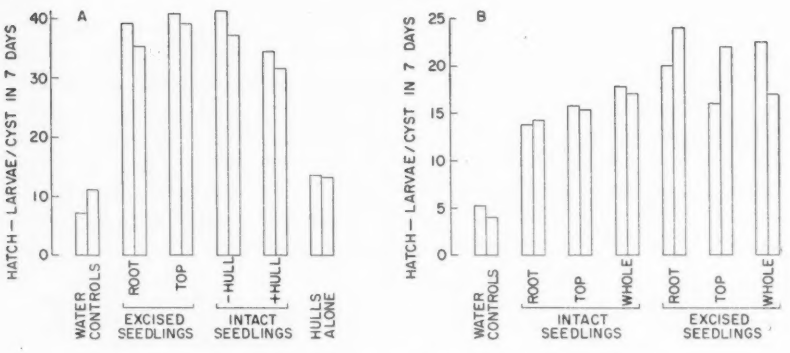


FIG. 7. Potency of leachate from various parts of young rape seedlings.

(12 ml. water per unit) as those from roots or tops alone. The results in Fig. 7B indicate that root and top again produced almost equally (either producing about half that from the whole seedling) and that cutting the seedling in two appeared to enhance production. The latter effect could be due to faster liberation of the hatching factor through the cut surface.

Production of Hatching Stimulant by Various Regions of the Seedling Root

Five portions, each 3–3.5 mm. long, were cut from the roots of twenty-four 2-day-old rape seedlings. The first section included the root tip and the portion of the root without root hairs. The root sections were leached in duplicate batches, each of 12 similar portions in 5 ml. of water for 1 hour. The duplicate samples of leachate thus produced were each diluted to 20 ml. (1.7 ml. per root portion) and tested on approximately 400 cysts. The results are presented in Fig. 8A.

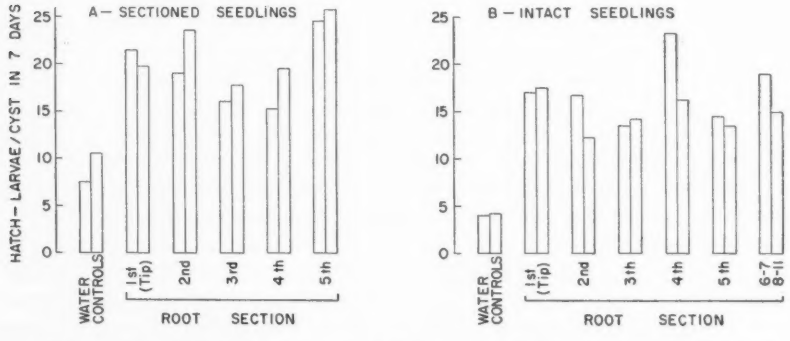


FIG. 8. Potency of leachate from various regions of the roots of intact and sectioned rape seedlings.

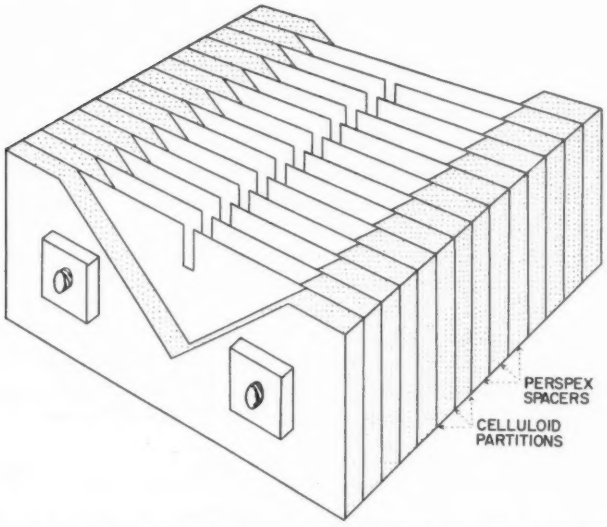


FIG. 9. Plastic holder used for leaching various regions of young intact seedlings.

In another experiment, intact seedlings were leached in special holders. The holder (Fig. 9) was built from a number of rectangular portions (2 in. $\times$ 1 in.) of $\frac{1}{8}$ in. Perspex sheet, each with a V-shaped section removed from one side. These were interleaved with rectangles of thin celluloid, the whole being bolted together firmly to form a series of watertight chambers each with a capacity of approximately 1.0 ml. The slits in the partitions, made with finely-pointed scissors, were just wide enough to hold the rootlet loosely. A small blob of vaseline was placed in the bottom of each slit and the seedling inserted with the root tip touching the back partition. The rootlet was pressed down gently into the vaseline in each slit, the slits then carefully sealed up with vaseline, and water pipetted into each chamber. The water was withdrawn 1 hour later. Duplicate samples of leachate (0.8 ml. per root portion), each from 12 seedlings, were produced from each region of the root. These were tested on batches of approximately 400 cysts. The results are shown in Fig. 8B.

From the results of these two experiments, it is clear that leachates from all parts of the root were stimulatory.

Concentration and Extraction Techniques

Two methods of concentrating the leachate have been described in the case of the golden nematode hatching factor. In one of these methods the factor was adsorbed on charcoal which was then eluted with aqueous acetone (2). The loss of activity by this method was high, however (5), and it was superseded by a vacuum distillation method (10, 5). In attempts to separate the active principle from contaminants in the leachate or its concentrates, three

main methods have been used with some success in the case of the golden nematode hatching factor (5). These are: extraction with phenol, extraction with 1-butanol, and ion exchange chromatography. These five techniques were tried in the case of the beet nematode factors.

Concentration by Vacuum Distillation

Root leachate from sugar beet growing in gravel was usually slightly alkaline. On concentration by vacuum distillation at 35°–40° C., the pH increased and the nematode-stimulating power was largely destroyed. When the leachate was adjusted to pH 6.0 by the addition of normal sulphuric acid (a technique previously used in the case of the golden nematode hatching factor (5)), there was no obvious loss of activity on concentrating 20-fold–40-fold. If overacidified, the leachate became more acidic on concentration and again some activity appeared to be lost. By progressively re-diluting correctly prepared concentrates, complete concentration–activity curves, such as that shown in Fig. 10A, were obtained. Somewhat similar curves were found for rape leachate concentrates (Fig. 10B).

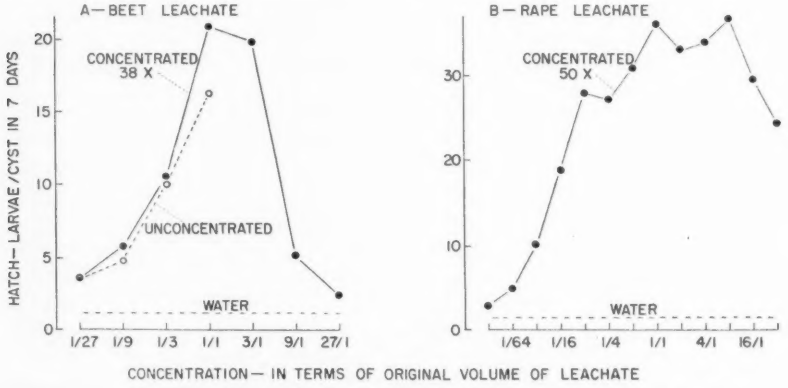


FIG. 10. Activity curves for leachate concentrated by vacuum distillation at 35°–40° C.

When samples of sugar beet root leachate at pH 6 were concentrated 20-fold, some material was precipitated. Such precipitates were relatively inactive, most of the activity being found in the filtrates which indeed were frequently more active than the original leachates. When these filtrates were concentrated a further 1-fold, however, much more of the activity appeared in the new precipitates, indicating that at the higher concentration some of the active material was taken out of solution.

Adsorption on Charcoal

An attempt was made to isolate the beet nematode hatching factors in sugar beet and rape root leachates by charcoal adsorption. One liter of leachate was shaken with 1 g. charcoal which was then recovered by filtration

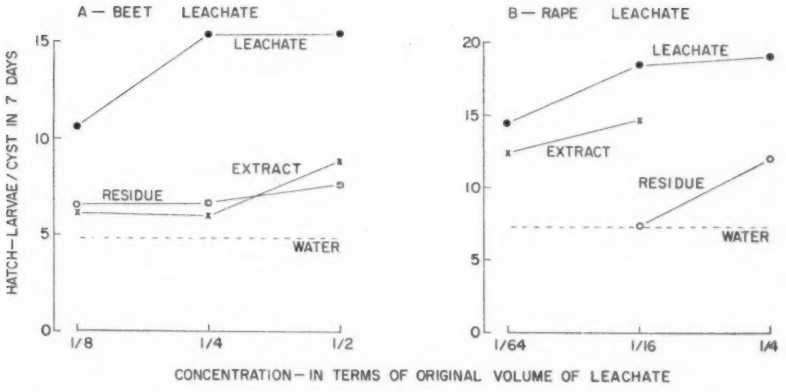


FIG. 11. Results obtained by extracting root leachates with charcoal and eluting with acetone.

and eluted by shaking with four 15-ml. aliquots of acetone–water (7:3) mixture. The four acetone–water extracts were pooled and the acetone evaporated by vacuum distillation at room temperature. Bio-assay tests were carried out on the untreated leachate, the leachate after extraction, and the extract. The results, Fig. 11, showed that most of the activity in the beet leachate was adsorbed on charcoal but very little recovered by elution with acetone. This confirms the earlier findings (4). Charcoal also removed the activity from rape leachate but in this case recovery was much better.

Extraction with Phenol

This method had been used successfully in the case of the golden nematode hatching factor by Carroll *et al.* (5), who found that when active extracts of potato or tomato root leachate at neutral pH were shaken with water-saturated phenol, the factor tended to pass into the phenol, while most of the impurities were left in the aqueous layer. On back-extraction of the phenol with water in the presence of excess diethyl ether, the factor passed back into the water which was found to contain most of the activity with only 5% of the total solids originally in the charcoal eluate. The method was used with equal success in the case of the beet and cabbage nematode hatching factors in black mustard root leachate, but was less effective when applied to the hop nematode factor in hop root leachate (4).

In a number of experiments, concentrated beet leachate was extracted with phenol and back-extracted as recommended (5), but the final extract was always inactive. Even when treble the quantity of phenol was used, the extract was still relatively inactive, some 50% of the activity being left behind in the concentrate (Fig. 12A). The same technique was applied to the beet nematode factor in rape root leachate with much more success, the phenol extract containing about 50% of the activity of the untreated leachate, which after treatment was quite inactive (Fig. 12B).

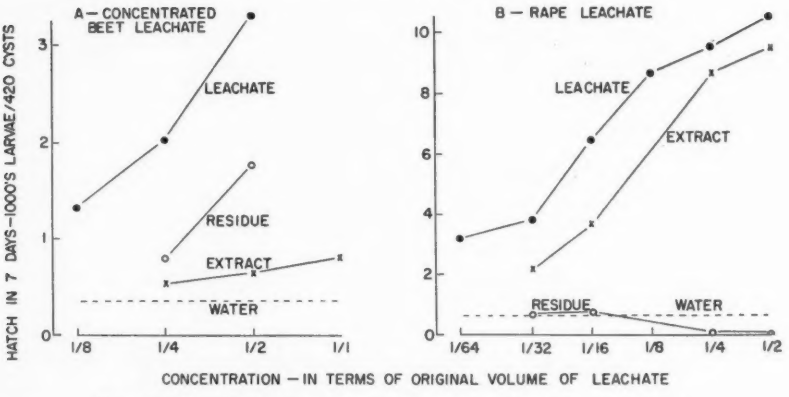


FIG. 12. Results obtained by extracting of root leachate with phenol.

Extraction with 1-Butanol

Samples of concentrated beet root leachate were adjusted to approximately pH 3 with dilute sulphuric acid and extracted by mixture with an equal volume of 1-butanol in a separatory funnel. The aqueous and butanol layers were concentrated by vacuum distillation at 35° C. and taken to dryness over potassium hydroxide and phosphorous pentoxide *in vacuo*. The residues were taken up in distilled water and assayed. The results showed the butanol extract to be relatively inactive. When small samples of the concentrated leachate were adjusted to pH 3 and extracted three times with an equal volume of butanol (i.e. using three times as much butanol as in the preceding experiments), most of the activity passed into the butanol layer. On similar extraction at pH 5, the butanol extract was relatively inactive, more of the activity remaining in the aqueous layer (Fig. 13).

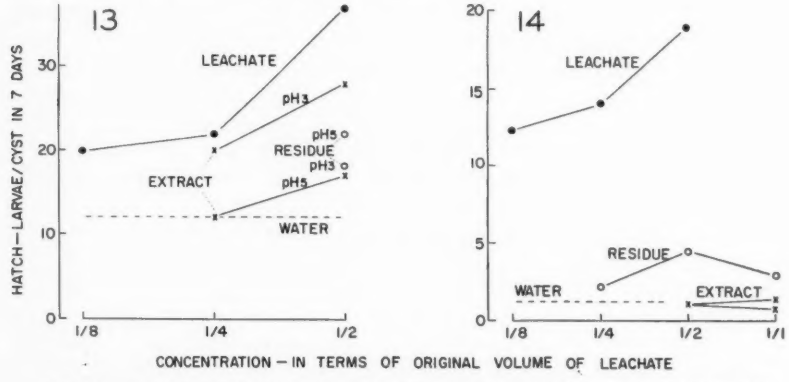


FIG. 13. Results obtained on extraction of concentrated beet root leachate with 1-butanol at two pH levels.

FIG. 14. Purification of root leachate by ion exchange.

The solubility of water in butanol is relatively high, being of the order of 15–20%. Therefore, the use of large volumes of butanol results in such a high proportion of the crude leachate or concentrate being taken up by the butanol as to render the process inefficient as a purification step.

Ion Exchange Chromatography

This method had been used by Carroll *et al.* (5) in purification of the golden nematode factor. They introduced active extracts of potato or tomato root leachates onto a column of Dowex 2-X anion resin in the formate form, then eluted the column successively with water, 1 *N* and 4 *N* formic acid, and collected the eluate as a number of fractions. When these fractions were freed from formic acid and bio-assayed, peak activity was seen to be concentrated in a few fractions, other fractions being relatively inactive.

The method, however, could not be applied in the case of the cabbage nematode factor in black mustard leachate, since this factor was destroyed by formic acid of the strengths used (4).

In a number of experiments, concentrated beet leachate was passed through ion exchange columns prepared as recommended and the columns eluted as recommended (5). Although most of the activity was removed from the concentrates by passage through the column, all the fractions eluted were equally inactive (Fig. 14). This was not due to destruction by the formic acid since experiments showed that the beet nematode factors from sugar beet and rape were quite stable to this acid.

Discussion

The beet nematode occurring in southwestern Ontario behaves similarly to that found in Europe (1, 6, 11, 21) in that hatching is greater in aqueous leachate from growing host plants than in water. Presumably such leachates contain chemical substances (hatching factors) which stimulate hatching.

Illumination and temperature had a pronounced effect on the potency of leachates obtained from around the roots of sugar beet plants. Short days (8–10 hours) favored production of more active leachates than did long days (14–16 hours). Moderate soil temperatures (60°–70° F.) were superior to either extreme (48° F. or 80° F.) although the precise optimum appeared to vary with other growing conditions. It is impossible, however, to generalize with respect to field behavior from these observations since activity, as suggested by Fenwick (16), may simply be related to root growth and hence subject to the environmental complex regulating plant vigor.

Duration of the leaching or immersion period also had a marked effect on the potency of the leachate (Figs. 2 and 5). When germinating rape seeds or the roots of older rape or sugar beet plants came in contact with excess water, apparently the hatching factor was released into the water relatively quickly at first, then progressively more slowly. This decrease in rate may have been due either to decreasing root aeration during immersion or to exhaustion of the reserves of hatching factor within the plant.

The potency of leachate from germinating rape seeds immersed in water was directly proportional to the concentration of the seeds in the water, at least within certain limits. By 'saturating' water with germinating seeds or young seedlings, leachate active at a dilution of 1/1,000,000 could be produced; presumably such leachate was much less contaminated with impurities, especially inorganic salts and decaying plant products, than that produced from older plants growing in soil or in gravel with nutrient solution. Produced in bulk, leachate from germinating seeds should, therefore, form a much better starting material for work on the chemistry of hatching factors.

Leachate from all parts of the germinating rape seedlings, except the seed coat, stimulated hatching. There was no evidence of any inhibitory region in the rootlet, corresponding to the repellent tip region described by Wieser (19) in dealing with the attractiveness of tomato rootlets to the root-knot nematode, *Meloidogyne hapla*. The attractant for the beet nematode, if any, in rape roots, however, may well be a different chemical substance to the hatching factor and may be distributed differently in the plant.

Root leachate from sugar beet growing in gravel can be concentrated 40-fold, without obvious loss of activity, by vacuum distillation. On further concentration some of the activity may be carried down with precipitated impurities, possibly by adsorption on these, as suggested by Janzen and Van der Tuin (12). The complete concentration–activity curve (Fig. 10A) shows that beet root leachate as collected was near the optimal concentration for stimulating hatch. Concentrations below this optimum caused progressively less hatch, the curves for concentrated and unconcentrated leachate being roughly similar and corresponding to Fenwick's 'dilution curve' for potato root leachate (8). Concentrations above the optimum also caused progressively less hatch, presumably owing to the presence of inhibitory material. Similar inhibition of hatching of the golden nematode by high concentrations of potato root leachate was noted by Hague (10), who suggested that the inhibition was associated with high concentrations of the hatching factor rather than with the presence of nutrient salts.

Results reported in this paper suggest that the observed inhibition (Fig. 10) was due to causes other than supraoptimal concentrations of the factor. Firstly, results of various experiments showed that a degree of purification, such as the precipitation of impurities during concentration, could result in enhanced activity. Secondly, leachate from germinating rape seeds could be produced capable of stimulating hatching at concentrations ranging from 1/1 to 1/1,000,000 (Fig. 6); in other words, this leachate was stimulatory over a far greater range of concentrations than that covered in the experiments represented by Fig. 10. Such leachate no doubt lacked many of the components (possible inhibitors) of leachate from plants grown in gravel with nutrient solution. Thirdly, root leachate could be produced in which hatching was less than that in water. This leachate evidently contained insufficient of the factor to stimulate hatch, yet it must have contained inhibitory material.

Thus the evidence on the whole suggests that the inhibition of hatching in concentrated root leachate was due not to high concentration of the hatching factor but to other components of the leachate.

The results of the various concentration and extraction procedures reported here must be regarded as definitely preliminary in character. They do suggest, however, that the factors produced by different host species may be somewhat different. Phenol extraction, for example, a procedure found by Carroll and his co-workers (4, 5) to remove the golden nematode factor from potato root leachates, was ineffective as a means of removing the beet nematode factor from beet root leachates but was effective when applied to rape root leachates.

Acknowledgments

This work was done during the tenure of a National Research Council postdoctoral fellowship held by the senior author. This assistance is gratefully acknowledged. The authors are also indebted to Mr. R. H. Mulvey, Science Service Laboratory, Ottawa, for help in obtaining supplies of infested soil.

References

1. Baunacke, W. Untersuchungen zur Biologie und Bekämpfung des Rübennematoden, *Heterodera schachtii* Schmidt. Arb. biol. Reichsanstalt Land. u. Forstwirtsch., Berlin-Dahlem, **11**, 185–288 (1922).
2. Calam, C. T., Raistrick, H., and Todd, A. R. The potato eelworm hatching factor. 1. The preparation of concentrates of the hatching factor and a method of bioassay. Biochem. J. (London), **45**, 513–519 (1949).
3. Calam, C. T., Todd, A. R., and Waring, W. S. The potato eelworm hatching factor. 2. Purification of the factor by alkaloid salt fractionation. Anhydrotetronic acid as an artificial hatching agent. Biochem. J. (London), **45**, 520–524 (1949).
4. Carroll, K. K. Purification and properties of eelworm hatching factors. 2. Hatching factors of the cabbage, hop and beet root eelworms (*Heterodera cruciferae*, *H. humuli* and *H. schachtii* respectively). Biochem. J. (London). Submitted for publication.
5. Carroll, K. K., Heyes, J. K., Johnson, A. W., and Todd, Sir Alexander. Purification and properties of eelworm hatching factors. 1. The hatching factor for the potato root eelworm *Heterodera rostochiensis* Woll.). Biochem. J. (London). Submitted for publication.
6. Emanuelsson, H. An investigation of the sugar-beet eelworm, *Heterodera Schachtii* (Schmidt). Socker Handl. H. **8**, 101–108 (1954).
7. Fenwick, D. W. Investigations on the emergence of larvae from cysts of the potato-root eelworm, *Heterodera rostochiensis*. 1. Technique and variability. J. Helminthol **23**, 157–170 (1949).
8. Fenwick, D. W. The bioassay of potato-root diffusate. Ann. Appl. Biol. **39**, 457–467 (1952).
9. Fenwick, D. W. The breakdown of potato-root diffusate in soil. Nematologica, **1** 290–302 (1956).
10. Hague, N. G. Concentration of potato root diffusate by vacuum distillation. Nature, **174**, 1018 (1954).
11. Hijner, J. A. De gevoeligheid van wilde bieten voor het bietencystenaaltje (*Heterodera Schachtii*). Mededel. Inst. Rationele Suikerproduct. **21**, 1–13 (1951).
12. Janzen, G. J. and Van der Tuin, F. The unknown hatching agent for the potato root eelworm. (Some purification aspects). Nematologica, **1**, 126–136 (1956).
13. Marrian, D. H., Russell, P. B., and Todd, A. R. The potato nematode hatching factor. 6. Attempts to prepare artificial hatching agents. Part II. Some active arylidene-β : γ-butenolides and related compounds Biochem. J. (London), **45**, 533–537 (1949).
14. Marrian, D. H., Russell, P. B., Todd, A. R., and Waring, W. S. The potato nematode hatching factor. 3. Concentration of the factor by chromatography. Observations on the nature of eclepic acid. Biochem. J. (London), **45**, 524–528 (1949).

15. MASSEY, L. M., JR. and NEAL, A. L. Investigations concerning the hatching factor of the golden nematode of potatoes, *Heterodera rostochiensis* Wollenweber. J. Wash. Acad. Sci. **43**, 396–401 (1953).
16. PETERS, B. G. Nematology Department. Rept. Rothamsted Expt. Sta. Year 1952, 93–99 (1953).
17. RUSSELL, P. B., TODD, A. R., and WARING, W. S. The potato eelworm hatching factor. 4. *Solanum nigrum* as a source of the potato eelworm hatching factor. Biochem. J. (London), **45**, 528–530 (1949).
18. RUSSELL, P. B., TODD, A. R., and WARING, W. S. The potato eelworm hatching factor. 5. Attempts to prepare artificial hatching agents. Part I. Some furan derivatives. Biochem. J. (London), **45**, 530–532 (1949).
19. WIESER, W. The attractiveness of plants to larvae of root-knot nematodes. I. The effect of tomato seedlings and excised roots on *Meloidogyne* hapla Chitwood. Proc. Helminthol. Soc. Wash. D.C. **22**, 106–112 (1955).
20. WILLIAMS, T. D. and WINSLOW, R. D. A synopsis of some laboratory techniques used in quantitative recovery of cyst-forming and other nematodes from soil. Soil Zoology. Proc. Univ. Nottingham 2nd Easter School in Agric. Sci. pp. 375–384. London: Butterworths Scientific Publications (1955).
21. WINSLOW, R. D. The hatching responses of some root eelworms of the genus Heterodera. Ann. Appl. Biol. **43**, 19–36 (1955).
22. WOOD, R. R. and SERRO, R. F. Identification of some materials in root exudates of nematode (*Heterodera schachtii* Schmidt) host plants. Proc. Am. Sugar Beet Technol. Year 1954, 271–275 (1954).

STUDIES OF THE AGING OF CONIDIA OF MONILINIA FRUCTICOLA (WINT.) HONEY

I. GERMINATION RATES AND LONGEVITY[1]

S. H. Z. Naqvi[2] and H. M. Good[3]

Abstract

Conidia of *Monilinia fructicola* (Wint.) Honey were stored 1–10 months at temperatures of 5°, 25°, and 35° C. and at constant relative humidities approximating 0, 15, 45, 75, and 90%. The germination of these spores was tested on potato dextrose agar containing 6% glucose, and curves of the percentage germination against time are given for representative samples.

A pronounced delay in germination developed under all conditions of storage. The rate at which this delay developed was virtually independent of the storage temperature. All spore samples gave 100% germination after 3 months but only those at 5° C. gave any germination after 10 months. Storage in a relative humidity of 75% gave the best preservation of spore vigor, there being evidence in other conditions of either high humidity or low humidity injury. Of the nutrient solutions tested, glucose was the only one which gave substantially better germination than water, the effect being most marked on spores stored for some months at high humidity. With glucose, such spores sometimes germinated faster than those stored at intermediate humidities.

Introduction

It is a common observation that the age of a sample of fungus spores affects both the percentage capable of germination and the time required for germination. However, little quantitative information is available on such effects. Many of the records which have been published deal with herbarium material which was kept dry or in sealed tubes (8, 10, 13) though not under controlled conditions. Others give only fragmentary data on the effects of controlled environments.

Anderson, Henry, and Morgan (2), working on the effects of temperature and humidity upon the viability of conidia of *Piricularia oryzae*, stated that conidia remained viable longest when stored under cool, dry conditions. Similar results were obtained by Lambert (11) working on the teliospores of *Puccinia graminis tritici*. Angell and Hill (4) observed that sporangia of *Peronospora parasitica* from cabbage, *Peronospora* species from rhubarb, and *Bremia lactucae* from lettuce remained viable for long periods under dry conditions. Similar results were obtained by Noble (15) on *Urocystis tritici*, by Rosen and Weetman (18) on *Puccinia coronata*, and by Piemeisel (17) on *Ustilago zeae*.

On the contrary, Hashioka (9) working on *Sphaerotheca fuliginea* and Angell and Hill (5) on *Peronospora tabacini* have shown that cool and moist

[1]Manuscript received May 9, 1957.
Contribution from the Department of Biology, Queen's University, Kingston, Ontario, and Department of Botany, University of Karachi, Karachi, Pakistan. This project was supported by a National Research Council grant to the junior author. The paper is part of a thesis presented by the senior author to Queen's University, Kingston, in partial fulfillment for the Degree of Doctor of Philosophy.
[2]Junior Lecturer, Department of Botany, University of Karachi, Karachi, Pakistan.
[3]Associate Professor of Biology, Queen's University, Kingston, Ontario.

conditions were best for the longevity of spores. Doran (7) stated that the longevity of aeciospores of *Cronartium ribicola* stored at 7° and 15° and 23° C. was unaffected by temperature. But when aeciospores were stored in moist air, they retained their viability longer than when stored in drier air. These results agree with those of Anderson (3) on the spores of *Cylindrocladium scoparium*.

Maneval (13) and Peltier (16) working on uredospores of *Puccinia graminis tritici* reported that low temperatures and intermediate humidities were best, if the spores were to be stored for some time.

In some of these studies a loss of viability was not clearly distinguished from a delay in germination. Nevertheless, the variable reactions indicate that fungus spores differ widely in their tolerance to drying and in their reaction to humidity in storage. More data on the effects of storage conditions upon spores are, therefore, needed before any generalization may be made on the effects of storage conditions on spores.

An attempt to evaluate the effects of temperature, humidity, and duration of storage separately also seems necessary. Accordingly, an investigation designed to measure the effects of these variables on spores and to give some information on the comparative physiology of young and old conidia was undertaken using conidia of *Monilinia fructicola* (Wint.) Honey. This fungus was selected because it combines ease of handling in the laboratory, abundant sporulation in culture, and importance as a pathogen.

The methods of growing and handling the spores and the effects of storage condition on viability and rate of germination are considered in this report, together with data on the effect of nutrients on germination. Correlated studies of water loss, dry weight loss, and changes in respiratory pattern with age will be presented later.

Material and Methods

The fungus was isolated from a diseased peach fruit bearing conidia of *M. fructicola*. The isolate was tested for its pathogenicity and a single spore culture was then made. Mass subcultures of this were used throughout these studies.

Since a considerable quantity of spores was needed, a number of media were tested for their capacity to produce spores abundantly. The media included malt, bactopeptone, Richard's, Czapek's, potato dextrose, and canned peach homogenate agars in a wide range of concentrations. The tests of spore production were run at 12°, 25°, and 35° C. Of the media tested, potato dextrose agar with added dextrose (potato 200 g., dextrose 60 g., agar 20 g., distilled water 1000 ml.) when incubated at 25° C. gave the best results and this medium was used throughout the experiments except where spores were obtained directly from rotted peaches for comparison with those grown in the laboratory. The pH of the media was approximately 6.0. While this potato dextrose substrate cannot be assumed to produce spores exactly comparable to those produced in nature (6), all comparisons are based on spores from similar sources.

The fungus was grown in 16 oz. medicine bottles in which some 50 ml. of medium was spread over the flat side of the bottle. Spores were spread as uniformly as possible over this surface with a dry needle. The fungus covered the whole surface in 10–12 days giving zonate sporing areas with comparatively narrow sterile bands between.

The spores were freed from the medium by adding approximately 30 ml. distilled water and brushing the colony gently with a very fine camel's hair brush. The contents of about 10 culture bottles were then decanted into a 200 ml. narrow-mouth centrifuge bottle and centrifuged for 15 minutes at 2000 r.p.m. The supernatant was poured off the spores, which were then placed on a weighed No. 4 Whatman filter paper disk in a small Seitz filter connected with a suction pump. Air, dried by passage through a calcium chloride tower, was drawn through the spores for 15 minutes by which time the spores appeared dry and the filter paper had become crisp. The whole time taken in this operation, from addition of water to drying the spores, was approximately 35 minutes. Spores prior to, and after, their immersion in water were tested for germination and there was no evidence that this period of immersion had any tendency to germinate the spores or that it affected their pattern of germination. The layer of spores approximately 25 mm. in diameter and 1 to 2 mm. thick remaining on the filter paper was held in an incubator at 25° C. for 12 hours to complete the drying. Spores prepared in this manner are referred to as "fresh".

Spores were stored in 1-qt. glass fruit jars. The filter paper bearing the spores was put on a small watch glass which was supported on a 100 ml. beaker. Each jar contained 250 ml. of a saturated salt solution having an excess of the solid phase of lithium chloride, potassium nitrite, sodium chlorate, zinc sulphate, or sodium phosphate (dibasic) for 15, 45, 75, 90, and 95% relative humidities respectively at 20° C. Anhydrous calcium chloride was used to give zero relative humidity. At higher or lower temperatures, the relative humidities differ slightly from those stated. The glass tops were placed on the jars without rubber rings and the jars were placed in constant temperature chambers at 5°, 25°, and 35° C.

Unless otherwise noted, germination was tested on the modified potato dextrose agar discussed above. During the course of the study it was found that pH's below 6.0 gave improved germination but the difference did not seem sufficient to warrant a change in medium. Comparative tests on distilled water and nutrient medium in Van Tieghem cells were done in same experiments.

For each test a small sample of spores was picked up with a dry needle and shaken onto an agar plate or transferred to a Van Tieghem cell, and a sample of 500 spores was examined for each determination of the percentage germination. The criterion for germination was emergence of a germ tube to a length exceeding its width. Counts were made from five sectors of the Petri dish. To give both the percentage germination and the rate at which germination

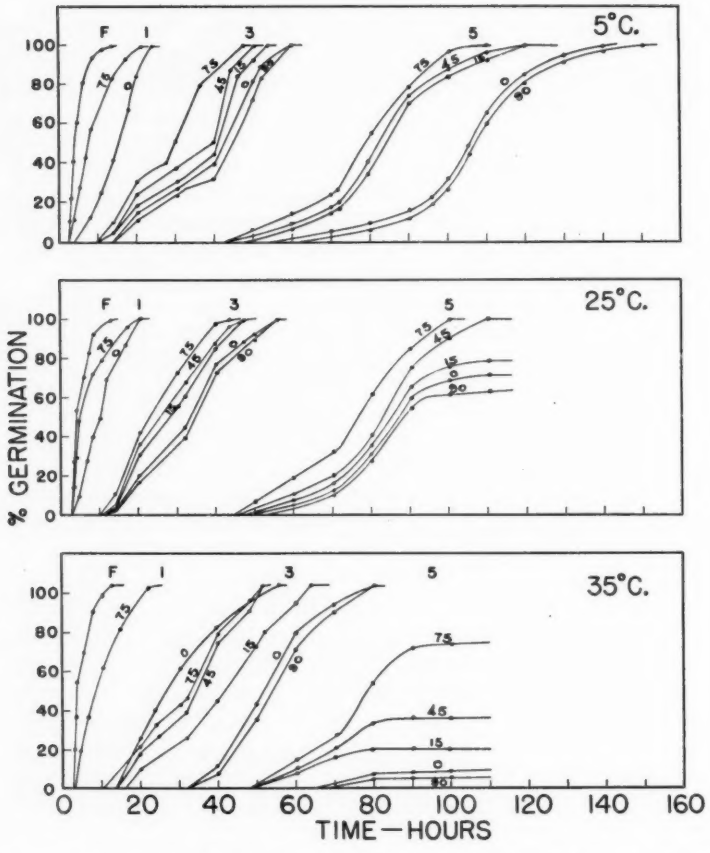


FIG. 1. Germination of stored spores of *Monilinia fructicola* (Wint.) Honey in relation to time from seeding on a potato dextrose agar containing 6% glucose. The temperature of storage is given at the top right corner of each rectangle, the period of storage in months as a number over each group of curves, and the humidity of storage as a number on the appropriate line.

occurred, counts were made at intervals until no further change occurred in the percentage of germinated spores. The times of these counts varied widely as shown in Figs. 1 and 2. The progress of germination with time was then graphed. This procedure had the advantage that it distinguished clearly between a delay in germination and a loss of viability. A number of earlier authors (4, 5, 9, 11) have determined percentage viability after an arbitrary time which appears, in the light of the data presented below, to have been far too short.

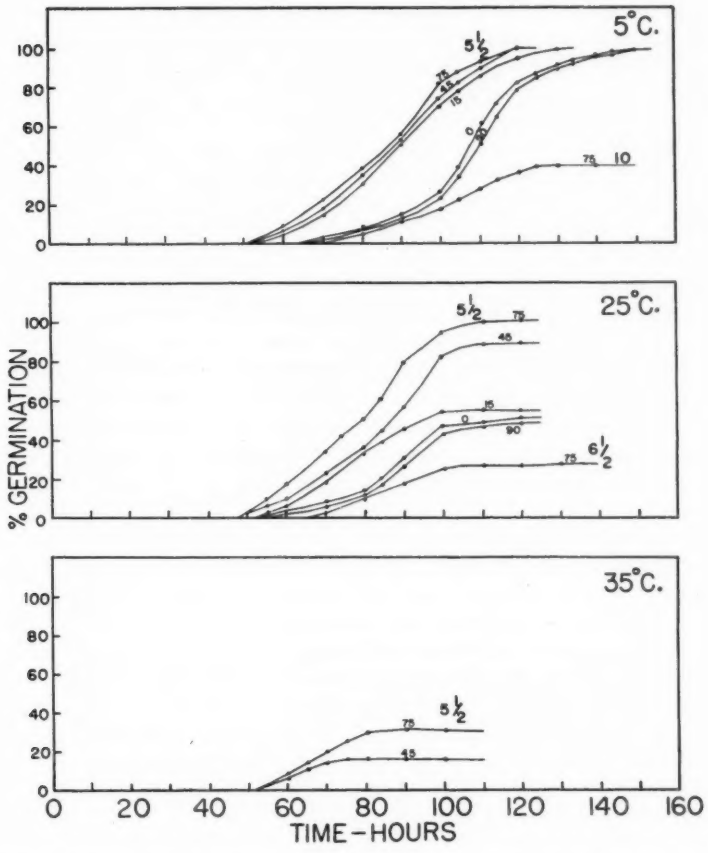


FIG. 2. Germination of stored spores of *Monilinia fructicola* (Wint.) Honey in relation to time from seeding on a potato dextrose agar containing 6% glucose. The temperature of storage is given at the top right corner of each rectangle, the period of storage in months as a number over each group of curves, and the humidity of storage as a number on the appropriate line.

Germination Rates and Longevity

1. Effect of Storage Conditions

A sample of spores was removed from each of the storage chambers, first at weekly, and then at monthly intervals, until no viable spores remained. The germination of each sample was determined as outlined under Materials and Methods. The data from these determinations showed the influence of humidity, temperature, and duration of storage upon the rate, uniformity, and maximum percentage of germination. Figs. 1 and 2 illustrate these relationships.

Data for several months have been omitted to prevent excessive crowding of the graphs. Curves for intermediate times may be interpolated without serious error. The whole series was run in duplicate, the trends being consistent for the two series.

Duration of Storage

The effects of duration of storage may be considered in relation to any of the conditions of temperature or humidity for which data are given in Figs. 1 and 2. For simplicity, discussion will be limited to the sample stored in a relative humidity of 75% at 25° C. Comparison of this sample with others may be readily made from the graphs of Figs. 1 and 2.

After one month in storage the spores germinated almost as quickly and just as completely as did fresh spores. Germination began at 3 hours, but some spores showed a delay so that complete germination required 20 hours. This is in contrast to 100% germination of fresh spores in 13 hours.

When the spores had been stored 3 months, there was a distinct delay in both the onset and completion of germination though there was no reduction in viability. Spores did not begin germinating till they had been 10 hours on the modified potato dextrose agar substrate and 100% germination was not reached till 44 hours had passed.

This delay in germination was even more pronounced in spores stored for 5 months. No germination took place within the first 44 hours yet at the end of 100 hours all the spores had germinated. The graph for spores stored 5 months illustrates both this pronounced delay in germination and the decreasing uniformity of the spores shown by the decreasing slope of the curve.

The curve for spores stored for 5½ months is essentially like that for spores stored for 5 months, though the delay in initiation of germination is even more pronounced. At this point many of the spores had reached the limit of their endurance. In the sample stored for 6½ months no germination occurred in less than 65 hours and only 26% of the spores were viable. No germination was obtained in samples held in storage more than 6½ months at this temperature and this humidity.

Relative Humidity of Storage Atmosphere

The data on germination of spores held at relative humidities of 0, 15, 45, 75, and 90% are given in Figs. 1 and 2. Since the effects of humidity were comparable at all the temperatures used and the data are presented in Figs. 1 and 2, only those at 25° C. will be discussed.

A distinctly harmful effect of both very high and very low humidities was shown. This was expressed after 1 month of storage when some of the severely dried spores (at 0% relative humidity) and the moist spores (at 90% relative humidity) were delayed in germination relative to those stored in a relative humidity of 75%. There was, however, no significant difference in the most vigorous spores of these samples, i.e. in the time required for the first germination. The effects of high and low humidities were more marked

after 3 months though again the onset of germination was not greatly affected by the humidity of storage except at 35° C. However, the germination graphs climb considerably more steeply for those spores stored at 75% relative humidity. Most of the spores stored 3 months at 90% or 0% relative humidity required about 10 hours more for germination than those stored at 75% humidity. This effect was almost equally marked in the very dry and very moist storage conditions.

As would be expected the effects of extremely humid conditions were much more marked after 5 months of storage. At this time there was a slight increase in the time required for the onset of germination at all temperatures, and a marked loss of viability at 25° and 35° C., particularly in the samples stored at 0% and 90% relative humidity. Spores stored 5 months at relative humidities of 0% and 90% were 71% and 66% viable respectively. After 5½ months those stored at relative humidities of 0% and 90% were only 50% and 47% viable respectively, and after 6½ months, only the sample stored at 75% humidity contained any viable spores (26%).

While humidity of the storage environment had a distinct effect on the germination of the spores, it is perhaps surprising that such extremes as were used did not have a more deleterious effect. The spores were shown to be extremely resistant to drying and to be essentially unaffected by months of storage at relative humidities within the very wide range of 15–75%.

Temperature

While the dormant spore is known to be much more resistant to heat injury than is growing mycelium, or an actively germinating spore, the processes which bring about senescence might nevertheless be expected to follow the pattern of physiological processes and to respond rather definitely to temperature. Failure to do so suggests that physical rather than physiological processes may be limiting the metabolism in the dormant dehydrated spore. While Figs. 1 and 2 show that the changes discussed under duration and humidity of storage developed most quickly at 35° C. followed by 25° C. and 5° C., the magnitude of the temperature effect was small. This was particularly true of the delay of germination. Spores stored for 5 months at 75% humidity and 5° C. took 39 hours longer than fresh spores to show the first stages of germination. Those stored 5 months at 25° C. and 75% humidity took only 42 hours longer. The changes responsible for the delay in germination of older spores appeared therefore to develop almost independently of temperature.

Temperature of storage had an appreciable effect on longevity of spores though the effect was not expressed within the first 5 months of storage. In a sample stored 5 months at 0% relative humidity and 5° C., the spores were 100% viable, whereas conidia stored at 25° C. were only 71% viable, and conidia in the same humidity at 35° C. were only 9% viable. However, the length of life of spores stored at 5° C. was less than double that of spores stored at 35° C. and only half again that of spores stored at 25° C.

2. Effects of Nutrients

Old spores have frequently been found to be more dependent than fresh spores on outside nutrients and the depletion of readily metabolized food reserves within the spore seemed a probable factor in this dependence and perhaps also in development of the delay in germination. Some investigation was therefore made of the influence of various dissolved substances on the germination of conidia of *M. fructicola*.

Stimulation of spore germination by various compounds is well known (12, 14). Of the materials of known constitution, carbohydrates such as glucose and sucrose have stimulated the germination most frequently (12, 19). Some amino acids and inorganic nitrogen have been successfully used for some species of *Glomerella*, *Penicillium*, and *Phycomyces* (12, 14) and some stimulation of spore germination by vitamins has been shown. Few fungi need dormancy breakers or such chemicals as 2,4-dinitrophenol (DNP), 2-methyl 1,4-naphthaquinone (MNQ), and coumarin, which were found to counteract the activity of inhibitors of germination of rust uredospores (1). In this study the influence of nutrients was of prime concern though some tests were run on vitamins and on a few other substances.

Tests were made by the hanging drop method in Van Tieghem cells. Initially fresh spores and spores stored for 4 months at 25° C. and at room humidity were used. Later, studies of the influence of glucose were made on spores stored at 0, 75, and 90% humidities and at 5° and 25° C. A control containing distilled water was included in each run.

When fresh spores were germinated in 0.1, 1.0, 3.0, and 6.0% glucose the patterns of germination were comparable to those in water. However, when 4-month-old spores were germinated in glucose solution, germination was more rapid and more complete than in distilled water. Of the four concentrations tested 6% glucose gave the greatest stimulation.

When glutamic acid, aspartic acid, leucine, proline, asparagine, glutamine, and ammonium nitrate were tested by germinating spores in solutions of 0.001, 0.01, 0.1, and 1.0% of these substances, none gave any significant increase in either rate or completeness of germination of fresh or 4-month-old spores.

Thiamine (0.001%), sodium thiocyanate (1.0 to 0.001%), ethylene chlorhydrin (1.0 to 0.001%), 2,4-dinitrophenol (1.0×10^{-5} molar), methyl naphthoquinone (2.8×10^{-6} molar), and coumarin (1.0×10^{-4} molar) were also tried. Only thiamine showed a stimulating effect and the improvement in germination was hardly significant.

Since 6% glucose gave the greatest, and indeed the only considerable effect, its action on spores of different ages was considered in further detail. Fresh and aged conidia (2, 4, and 5 months) stored at 25° C. and room humidity were germinated at 25° C. on 6% glucose and in distilled water. The addition of sugar did not change the rate of germination of fresh or 2-months-old conidia, but older conidia showed faster germination. For example, 2-month-old

spores required 24 hours for 50% germination in 6% glucose and 27 hours for 50% germination in water. Five-month-old conidia required 58 and 80 hours for 50% germination in 6% glucose and water respectively.

Since the effect of glucose seemed probably related to food depletion during storage, a further test was made of the effect of glucose on spores stored at 5° C. in humidities of 0, 75, and 90%. Under these conditions the spores vary in moisture content and hence in metabolic activity, those in 90% relative humidity being most active. The details of this variation will be reported later.

Spores stored at only the one temperature were available for this study. Germination tests were made at 25° C. A control was run on distilled water. The results of these tests are shown in Fig. 3.

Spores stored in a relative humidity of 75% and sown in distilled water began to germinate at about 60 hours while those stored at 0% and 90% required 76 and 88 hours respectively. After 120 hours those which had been stored at 75, 0, and 90% humidities showed 65, 15, and 10% germination respectively. Since later observations showed no increase these values were also taken as the percentages of viable spores. Conidia from the same samples germinated on 6% glucose began to germinate appreciably sooner and finally showed higher percentages of germinated spores. The values for these may be seen in Fig. 3. The percentage of spores appearing viable increased over those determined on water rising from 65 to 75%, for the sample stored at 75% relative humidity, from 15 to 30% of those stored at 0% relative humidity, from 10 to 40% for those stored at 90% relative humidity.

The glucose-induced increase in the rate of germination of the spores stored at a relative humidity of 90% was very marked. Moreover, in this sample it

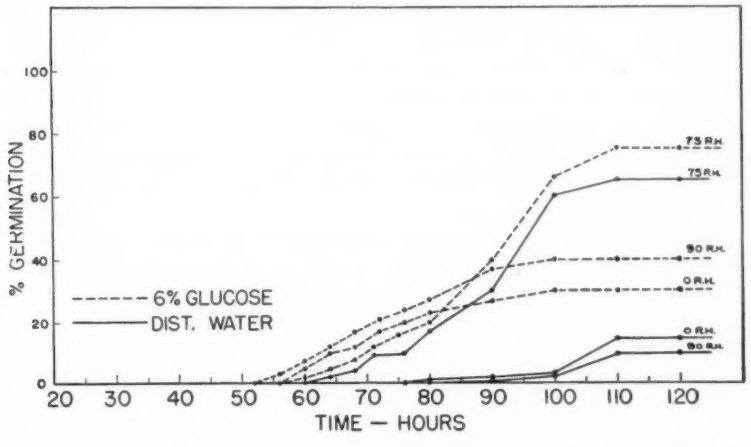


FIG. 3. Influence of 6% glucose on the germination and viability of *M. fructicola* conidia stored for 9 months at 5° C. and at relative humidities 0, 75, and 90%.

was sufficient to give a slightly higher rate of germination and final percentage germination with spores stored at 90% relative humidity than was shown by the sample stored at 0% relative humidity. This was also apparent in certain other samples although it does not show in any of those graphed in Figs. 1 and 2. Apparently storage at high humidity caused an earlier death of many spores than did storage at lower relative humidities but also provided for a more rapid germination of at least some of the spores when these were given the benefit of a glucose medium (Fig. 3). Unfortunately the return of the senior author to duties in Pakistan prevented further exploration of this interesting if somewhat inconsistent observation.

Discussion

Conidia of *M. fructicola* showed very considerable resistance to desiccation during storage in a fairly wide range of temperatures. Indeed the drying which conidia withstood without injury was more drastic than would be encountered in nature. It seems probable therefore that dehydration is not a significant factor in the inactivation of these spores.

Very moist conditions were more harmful than very dry conditions and conidia in nature must be subjected to frequent periods of very high humidities. Senescence of conidia in nature would therefore be expected to be influenced more by the periods of high humidity than by desiccation, and the effects of periods at humidities nearly adequate for germination should be determined. Unfortunately, the effects of fluctuating conditions lay outside the scope of this study.

As has been observed frequently, old conidia germinate slowly. It is interesting, however, that the delay may be so great before there is any change in the percentage of viable spores. Fresh spores began germinating regularly in 3–4 hours but stored spores developed a lag of some 36 hours before there was any loss of viability. In some cases onset of germination was delayed as much as 60 hours in samples of spores which later showed 100% germination.

The delay in germination which developed during storage was surprisingly independent of temperature. This suggests that the metabolism of the dormant spore is controlled, at least in part, by physical limitations probably imposed by the extreme dehydration of the cytoplasm. This may be seen from the curves in Fig. 1.

The marked advantage to the aged spore of being in contact with a considerable concentration of sugar is probably significant in brown rot where injuries may have produced an exudate with a high sugar content. In storage, long periods of high humidity coupled with available nutrient may well render effective spores which could not infect under field conditions where periods of adequate humidity are not likely to obtain for the 48 hours or more required for germination of old spores.

It is surprising to find that the various amino acids gave no stimulation of spore germination especially when evidence which will be presented later showed that proteins and amino acids play an important part in spore

metabolism. The carbohydrate reserves found in the spore were, on the other hand, negligible. Perhaps it is because the spore is rather poor in carbohydrates that glucose appeared to have an almost specific effect in stimulating germination. The role of other sugars has, however, not been investigated beyond determining that glucose was better than sucrose for growth of the fungus.

References

1. ALLEN, P. J. The role of self inhibitor in the germination of rust uredospores. Phytopathology, **45**, 259–266 (1955).
2. ANDERSON, A. L., HENRY, B. W., and MORGAN, T. L. The effect of temperature and relative humidity upon the viability of the conidia of *Piricularia oryzae*. Phytopathology, **38**, 574 (1948).
3. ANDERSON, P. J. Rose canker and its control. Mass. Agr. Expt. Sta. Bull. **183**, 11–46 (1918).
4. ANGELL, H. R. and HILL, A. V. The longevity of conidia of certain fungi (Peronosporales) under dry conditions. J. Council Sci. Ind. Research, Australia, **4**, 178–181 (1931).
5. ANGELL, H. R. and HILL, A. V. Blue mold of tobacco: longevity of conidia. J. Council Sci. Ind. Research, Australia, **4**, 181–184 (1931).
6. DARBY, R. T. and MANDELS, G. R. Effect of sporulation and age on fungus spore physiology. Plant Physiol. **30**, 360–366 (1955).
7. DORAN, W. L. Effect of temperature and internal factors on germination of fungus spores. Bull. Torrey Botan. Club, **49**, 313–336 (1922).
8. FISCHER, G. W. The longevity of smut spores in herbarium specimens. Phytopathology, **26**, 1118–1127 (1936).
9. HASHIOKA, Y. Relation of temperature and humidity to *Sphaerotheca fuliginea* with special reference to germination, viability and infection. Trans. Natural Hist. Soc. Formosa, **27**, 129–145 (1937).
10. HESSELTINE, C. W. Viability of some mould cultures. Mycologia, **39**, 126–128 (1947).
11. LAMBERT, E. B. The relation of weather to the development of stem rust in Mississippi Valley. Phytopathology, **19**, 1–71 (1929).
12. LIN, C. K. Nutrient requirements in germination of the conidia of *Glomerella cingulata*. Am. J. Botany, **32**, 296–298 (1945).
13. MANEVAL, W. E. The viability of uredospores. Phytopathology, **14**, 403–407 (1924).
14. MARLOTH, R. H. The influence of hydrogen ion concentration and of sodium bicarbonate and some related substances on *Penicillium italicum*, *P. digitatum*. Phytopathology, **21**, 169–198 (1931).
15. NOBLE, R. J. Studies on the parasitism of *Urocystis tritici* Koern. The organism causing flag smut of wheat. J. Agr. Research, **27**, 451–489 (1924).
16. PELTIER, G. L. A study of the environmental condition influencing the development of stem rust in the absence of alternate host. Nebraska Agr. Expt. Sta. Research Bull. **22**, 15 (1922).
17. PIEMEISEL, F. J. Factors affecting the parasitism of *Ustilago zeae*. Phytopathology, **7**, 294–407 (1917).
18. ROSEN, H. P. and WEETMAN, L. M. Longevity of uredospores of crown rust of oats. Arkansas Agr. Expt. Sta. Bull. **391**, 3–20 (1940).
19. UPPAL, B. N. Spore germination of *Phytophthora infestans*. Phytopathology, **14**, 32–33 (1924).

QUANTITATIVE DETERMINATION OF VIABILITY OF POTATO RING ROT BACTERIA FOLLOWING STORAGE, HEAT, AND GAS TREATMENTS[1]

LLOYD T. RICHARDSON

Abstract

A method for determining quantitatively the viability of *Corynebacterium sepedonicum* was developed for use in eradication studies on bacterial ring rot of potatoes. Drops of bacterial suspension dried on glass or jute fibers impregnated with the organism were used as test materials. The number of viable bacteria in a population was derived from the lag period in its time–growth curve based on turbidity development in a liquid medium. It was demonstrated that ring rot bacteria on dry jute can survive storage at 0° C. for 6 months with no loss in viability. Although viability decreases with time at higher temperatures, mortality is not complete after 6 months at 28° C. The thermal death points of the organism for 10 minute exposures were found to be 55° C. in liquid medium and 125° C. in dry air for bacteria on glass and 150° C. in dry air for bacteria on jute fibers. The minimum gas dosage found to achieve complete kill of ring rot bacteria on dry jute fibers in 18 hours was 2% ethylene oxide by volume of air or 20% methyl bromide.

Introduction

The spread of bacterial ring rot in seed potatoes may be prevented by the eradication of the causal organism, *Corynebacterium sepedonicum* (Spiek. and Kott.) Skapt and Burkh., from potential sources of contamination. Many treatments have been tested for this purpose, including the use of liquid chemical disinfectants (1), gaseous fumigants (4, 6), antibiotics (11), steam heat (9), infrared heat (5), and ultraviolet rays, X-rays, and nuclear radiations (10). The development of a successful control program has been hampered for want of a practical method of accurately evaluating the effectiveness of any treatment within a reasonable period of time.

In most cases treatments have been evaluated on the basis of disease symptoms developing in potato plants grown from inoculated tubers (3, 8, 9, 10, 11). Young tomato plants have also been used as indicators (1, 6). Both methods involve a waiting period of several months and give results which are extremely variable. MacLachlan *et al.* (3, 4) evaluated infrared heat and gas treatments of infested jute in 8 days by culturing surviving bacteria on a solid nutrient medium. Katznelson and Sutton (2) compared the bacteriostatic effects of several antibiotics and quaternary ammonia compounds *in vitro* on the basis of complete inhibition of growth after 24 hours' incubation in a liquid medium.

While these methods may indicate the degree of treatment required to inhibit the organism completely, none of them show the relative effectiveness of varied rates of treatment. The procedure developed in the present study makes possible a quantitative determination of the degree of viability of a population of ring rot bacteria from the growth rate in liquid culture as indicated by turbidity.

[1]Manuscript received May 10, 1957.
Contribution No. 91 from the Science Service Laboratory, Canada Department of Agriculture, London, Ontario.

Methods

In order to use turbidity as a measure of bacterial growth it is essential to have a completely transparent liquid medium which will permit rapid multiplication of bacterial cells. Since none of the liquid media recommended for the growth of *C. sepedonicum* met these requirements a number of combinations of ingredients known to promote its growth were tested. The following formulation was finally adopted as a result of these tests:

yeast extract	1 g.
casein hydrolyzate	1 g.
dextrose	1 g.
calcium pantothenate	0.1 g.
water	1000 ml.

This medium is pale amber in color, supports rapid growth of the organism, and is completely free from sediment. Without adjustment the pH is approximately 7.0 after autoclaving.

The medium was dispensed in 10-ml. quantities into 6 by $\frac{3}{4}$ in. culture tubes. After inoculation these were incubated at room temperature in a sloping position on a reciprocal shaker making 120 excursions per minute. As soon as visible growth appeared, the turbidity of the liquid in each tube was measured daily by means of a photoelectric nephelometer which permitted direct readings in $\frac{3}{4}$-in. test tubes. The purity of any questionable culture was checked by microscopic examination of Gram-stained smears.

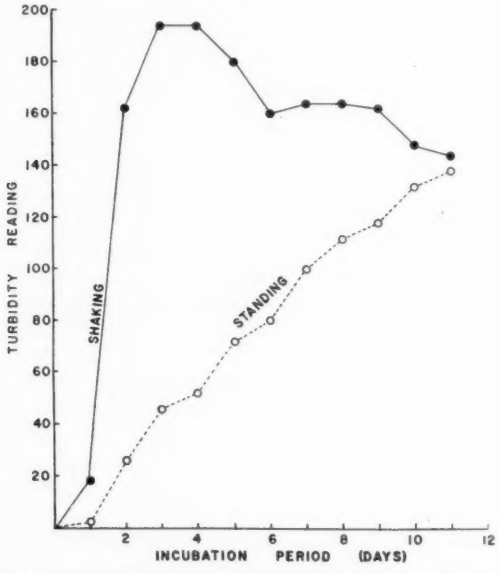


FIG. 1. Growth curves of shaking and stationary liquid cultures of *Corynebacterium sepedonicum* derived from turbidity measurements.

The increased aeration of the cultures achieved by shaking accelerated the growth of the organism, as shown by the growth curves for shaking and standing cultures in Fig. 1.

Bacterial populations were prepared for treatment in two ways. In the first, used when necessary to exclude the possibility that results might be affected by the presence of substrate, bacteria were removed from liquid cultures by centrifuging and were resuspended in sufficient sterile distilled water to give a fixed turbidity reading. Uniform drops (0.1 ml.) of this suspension were then pipetted onto small pieces of glass cover slips and allowed to dry at room temperature in sterile petri dishes. The second material, for use in studies of treatments to disinfest contaminated potato bags, was prepared in the following way to simulate natural conditions. Five strands of jute 2 in. long cut from a potato bag were laid on the surface of a potato slice in a petri dish lined with moist filter paper. After it was autoclaved for 20 minutes at 15 lb. pressure and allowed to cool, 1 ml. of bacterial suspension in liquid medium was pipetted over the surface of each potato slice. When a yellow film of bacterial growth had developed over the surface the jute strands were transferred to sterile petri plates and allowed to dry at room temperature. Replicate fibers were taken from different potato slices to reduce variability.

After treatment each sample on glass or on jute was transferred to an individual tube of liquid medium and incubated. Being transparent, the glass carriers did not interfere with turbidity reading and so were left in the tubes throughout the growth period, but the jute strands were removed before the first turbidity reading was taken.

Determination of Number of Viable Bacteria in Inoculum

An experiment was conducted to establish the relationship between the number of viable bacteria in the inoculum and the rate of turbidity development in liquid cultures. A series of 1:10 dilutions in liquid medium were made starting with a culture which had attained maximum growth. One milliliter from each dilution was then transferred to each of duplicate tubes of medium, using a fresh pipette for each transfer. The growth curves obtained from daily turbidity readings are shown in Fig. 2. These indicate a lag period, varying with the concentration of bacteria in the inoculum, before measurable growth occurred. No growth developed from inoculum diluted beyond 1×10^{-11}. If we assume that at this dilution end point the inoculum contained a single bacterial cell, then the concentration of bacteria in the original tube would be in the order of 10^{11} per ml. It is apparent that only the final stages of growth are visible since only the first dilution had measurable turbidity before incubation. Thus the duration of the lag period as well as the actual turbidity readings must be taken into account in assessing concentrations of viable bacteria by this method.

A straight-line calibration curve (Fig. 3) for converting lag periods to numbers of viable bacteria in the original inoculum was derived by plotting

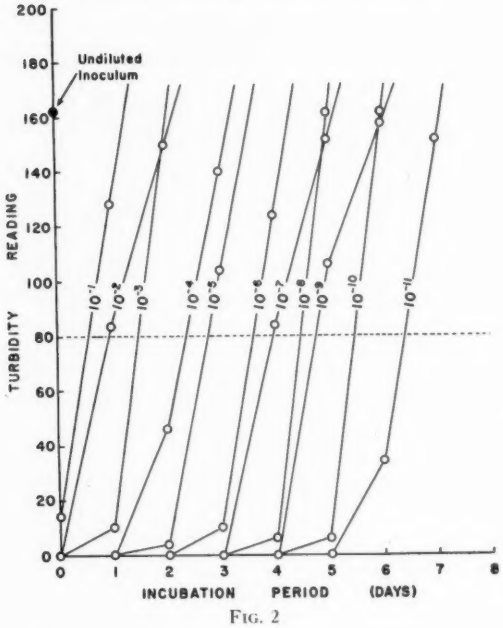

Undiluted Inoculum
TURBIDITY READING
INCUBATION PERIOD (DAYS)
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
10^{-8}
10^{-9}
10^{-10}
10^{-11}

FIG. 2

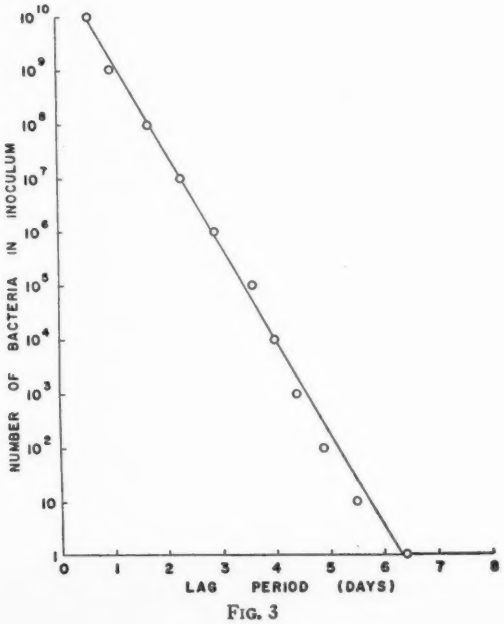

NUMBER OF BACTERIA IN INOCULUM
LAG PERIOD (DAYS)

FIG. 3

the time, in days, when the growth curves in Fig. 2 reached 80, the mid-point in turbidity, against the number of bacterial cells in the inoculum. The number of viable bacteria in an unknown sample can thus be determined from the lag period found from its time–growth curve.

Longevity of *C. sepedonicum* on Jute

The liquid culture method was applied to gain further information as to the longevity of *C. sepedonicum* on jute under conditions comparable to those under which contaminated potato bags are likely to be stored. Dry infested jute fibers were kept for 6 months in sterile petri dishes at 0°, 7°, 16°, 22°, and 28° C. At monthly intervals five replicate fibers from each lot were cultured to determine the numbers of surviving bacteria. The viability data presented in Table I indicate that there was no loss in viability of bacteria in 6 months on fibers held at 0° C. At the higher temperatures the viability decreased with storage time and the rate of decline increased as the storage temperature increased. Viability was not completely lost in 6 months even at 28° C. where an average of 1000 bacteria per fiber survived.

TABLE I

EFFECT OF TEMPERATURE AND STORAGE PERIOD ON VIABILITY OF RING ROT BACTERIA ON DRY JUTE FIBERS

Storage period months	Storage temperature				
	0° C.	7° C.	16° C.	22° C.	28° C.
0	400*	400	400	400	400
1	700	300	400	300	300
2	700	200	300	15	2
3	700	400	300	4	1
4	1000	300	400	2	0.15
5	200	—	4	0.2	0.07
6	700	70	2	0.003	0.001

*Number of viable bacteria per fiber in millions (average of five replicates).

Thermal Resistance of *C. sepedonicum*

The thermal resistance of *C. sepedonicum* under various conditions was assessed by determining the viability of populations of the organism following exposure on glass and on jute in liquid medium and in air to a range of temperatures.

To determine the thermal death point of the organism when wet, uniform smears on cover-slip glass were placed in tubes of the liquid medium preheated to the required temperature in a water bath. After a 10 minute exposure the

FIG. 2. Growth curves of liquid cultures of *C. sepedonicum* showing the effect of inoculum concentration on the length of the lag period (numbers on curves indicate dilution of inoculum).

FIG. 3. Relation between number of viable bacteria in inoculum and lag period (see Fig. 2).

TABLE II

EFFECT OF TEMPERATURE ON VIABILITY OF RING ROT BACTERIA ON GLASS IN LIQUID MEDIUM (10 MINUTE EXPOSURES)

Temperature	Number of surviving bacteria
Control	200,000,000
50° C.	4,000,000
51° C.	2,000,000
52° C.	300,000
53° C.	1,000
54° C.	10
55° C.	0

tubes were removed, cooled rapidly, and incubated. No loss of viability was detected at 45° C. or below, whereas inhibition of growth was complete at 55° C. The viability of cultures exposed for 10 minutes to temperatures between 50° and 55° C. may be seen in Table II. The thermal death rate in liquid medium at a sublethal temperature, 50° C., is represented in Fig. 4, where the log of the number of survivors is plotted against exposure times up to 90 minutes.

The thermal resistance of bacteria multiplied from the survivors of a 10-minute exposure to 54° C. was compared with that of the original culture

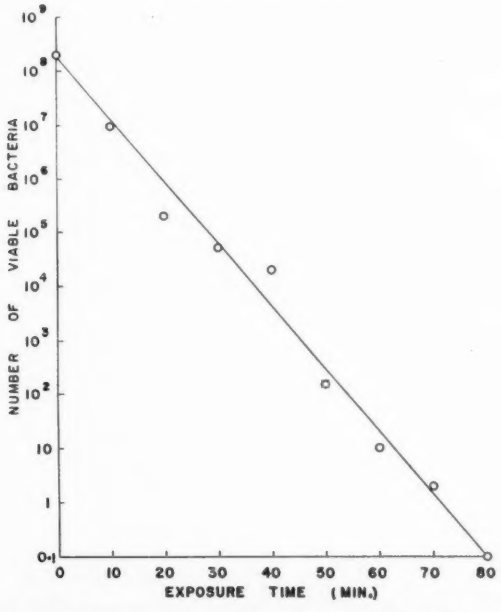


FIG. 4. Rate of loss of viability of ring rot bacteria exposed to 50° C. in a liquid medium.

by exposing smears prepared from both sources to 54° and 55° C. for 10 minutes. The survivors from this second 54° treatment were also multiplied and again exposed to 54° and 55° for 10 minutes. The viability of all these populations is shown in Table III. Evidence of increased inherent thermal resistance in bacteria surviving the first heat treatment is shown by the greater proportion of survivors from subsequent 54° treatments; moreover, the thermal death point was evidently raised, for some bacteria were able to survive 55° C. for 10 minutes.

TABLE III

A COMPARISON OF THERMAL RESISTANCE OF RING ROT BACTERIA SURVIVING HEAT TREATMENTS WITH THAT OF THE ORIGINAL CULTURE

Previous heat treatment	Present heat treatment	No. of survivors
None	None	40,000,000
	10 min. at 54° C.	1
	10 min. at 55° C.	0
10 min. at 54° C.	None	80,000,000
	10 min. at 54° C.	15,000
	10 min. at 55° C.	1,000
10 min. at 54° C. (twice)	None	3,000,000
	10 min. at 54° C.	30,000
	10 min. at 55° C.	500

TABLE IV

EFFECT OF TEMPERATURE ON THE VIABILITY OF RING ROT BACTERIA ON GLASS AND ON JUTE FIBERS IN DRY AIR (10-MINUTE EXPOSURES)

Temperature (Centigrade)	Average number of viable bacteria per sample	
	On glass	On jute
Control	300,000,000	5,000,000,000
100°	2,000,000	—
105°	50,000	—
110°	70,000	—
115°	20,000	—
120°	150	2,000,000,000
125°	0	440,000,000
130°	—	80,000,000
135°	—	1,000,000
140°	—	2,000,000
145°	—	45,000
150°	—	0

In tests of the resistance of ring rot bacteria to dry heat smears on both glass and infested jute fibers were used. These test materials were placed in sterile empty test tubes preheated to the required temperature. After exposure for 10 minutes the tubes were removed, cooled rapidly, and provided with liquid medium. The numbers of bacteria surviving following exposure to a range of temperatures are tabulated in Table IV. As was expected much higher temperatures were required to kill the bacteria with dry heat than

with moist heat. On glass the thermal death point for a 10 minute exposure in air was 125° C. as compared with 55° C. for the same samples in liquid medium. Still higher temperatures were required to kill the organism on the jute substrate, probably owing to a protective effect although greater numbers of cells were involved. While complete kill was achieved in 10 minutes at 150° C. with the isolate used in all of these tests, a few bacteria survived even this temperature when three of four cultures isolated from other sources were tested in the same way.

Evaluation of Toxicity of Gaseous Fumigants

The liquid culture method was used to evaluate the toxicity to *C. sepedonicum* of certain gases which might be used to fumigate infested potato bags. Both impregnated jute fibers and bacteria dried on glass were used as test materials. These were enclosed in sterile petri dishes with lids slightly raised and exposed to a series of dosages of the fumigants at atmospheric pressure in vacuum desiccators, following the procedure of Sampson and Ludwig (7). The samples were transferred to tubes of liquid medium immediately after treatment and incubated.

The results of tests with ethylene oxide and methyl bromide are presented here as examples of the applicability of the method. Samples were exposed 18 hours to mixtures of ethylene oxide from 1% to 2.5% by volume in air or methyl bromide from 2.5% to 20%. The relative humidity was approximately 30%. The effect of these gases on the viability of *C. sepedonicum* is shown in Fig. 5 where the number of survivors is plotted against dosage using log scales on both axes. It should be noted that higher dosages were required to achieve complete mortality in other tests where the fibers carried heavier concentrations of inoculum.

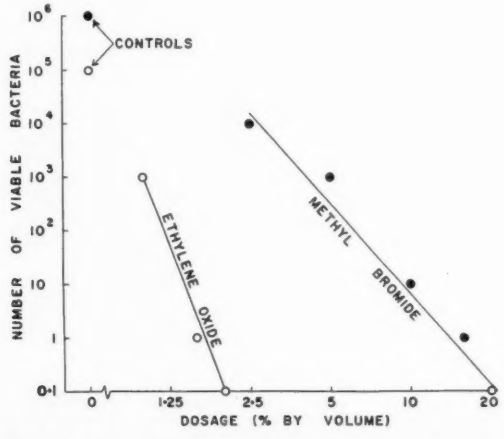


FIG. 5. Effect of various dosages of ethylene oxide and methyl bromide on viability of ring rot bacteria on dry jute fibers.

Discussion

The results of this study indicate that the lag phase in the growth rate of *C. sepedonicum* in a liquid medium can be used as an index of the number of viable bacteria in the inoculum. Periodic measurement of turbidity in liquid cultures provides a precise practical method of determining quantitatively within a few days the viability of any pure population of the organism and permits the evaluation of graded eradicatory treatments on the basis of the number of survivors which may vary from a trace to many million.

Using this method the longevity of ring rot bacteria on dry jute at different temperature levels was demonstrated. Virtually no loss in viability occurred in six months at temperatures at which potatoes are normally stored while even at 28° C. (82° F.) an average of 1000 bacteria per 2-in. jute strand survived. These results substantiate the reports (4, 8) that ring rot bacteria on jute potato bags remain viable and infective after seven months at normal storage temperatures. Contaminated potato bags, therefore, constitute a potential source of ring rot infection of seed potatoes.

The thermal resistance of *C. sepedonicum* shown by the liquid culture method is comparable to that of most bacterial plant pathogens. Complete kill of ring rot bacteria on glass was achieved in 10 minutes at 55° C. in liquid medium or 125° C. in dry air though 150° C. in air was required to kill bacteria on dry jute fibers. MacLachlan and Racicot (5) recovered no ring rot bacteria on agar plates from contaminated jute squares exposed 90 seconds to 218° C. in an infrared oven.

The relative effectiveness of graded dosages gaseous fumigants against ring rot bacteria was also demonstrated by the liquid culture method. In the examples cited there were no survivors on jute fibers exposed for 18 hours to mixtures of 2% ethylene oxide by volume in air or 20% methyl bromide. MacLachlan *et al.* (4) reported complete mortality of ring rot bacteria on jute squares after 16 hours in 100 mg. ethylene oxide per liter (5.5% by volume) at atmospheric pressure but no effect from 150 mg. methyl bromide (4% by volume) in that time. Mai *et al.* (6) found no reduction in ring rot infection of tomato plants inoculated with contaminated tooth picks treated for 16–20 hours in 60 lb. methyl bromide per 1000 cu. ft. (25% by volume). Although ethylene oxide is much more active than methyl bromide in killing ring rot bacteria it should be remembered that in practice the efficiency of a fumigant for disinfecting potato bags will be determined by other factors (e.g. penetration and sorption) in addition to inherent toxicity to the organism.

References

1. Gozenback, C. A biological method of testing chemicals for the control of the ring rot pathogen (Abstract) Phytopathology, **40**, 10 (1950).
2. Katznelson, H. and Sutton, M. D. Inhibition of plant pathogenic bacteria *in vitro* by antibiotics and quaternary ammonium compounds. Can. J. Botany, **29**, 270-278 (1951).
3. Knorr, L. C. Field testing of disinfectants for the control of potato ring rot. Bacteria on wooden and metallic surfaces. Am. Potato J. **24**, 141-150 (1947).

4. MacLachlan, D. S., Monro, H. A. U., Racicot, H. N., and King, J. E. Fumigation of potato bags with toxic gases for the control of bacterial ring rot of potato. Can. J. Agr. Science, **33**, 132-140 (1953).
5. MacLachlan, D. S. and Racicot, H. N. Some methods of used bag disinfection in potato ring rot control. Quebec Soc. Protection of Plants, 32nd Ann. Rept. 1950.
6. Mai, W. F., Lear, B., and Boothroyd, C. W. Ineffectiveness of methyl bromide for killing the causal organisms of ring rot and seed piece decay of potatoes. Plant Disease Reptr. **35**, 356-357 (1951).
7. Sampson, R. E. and Ludwig, R. A. Laboratory studies on the evaluation and activity of antifungal fumigants. Can. J. Botany, **34**, 37-43 (1956).
8. Starr, G. H. The longevity of *Corynebacterium sepedonicum* on potato bags when placed under different environmental conditions. Am. Potato J. **24**, 177-179 (1947).
9. Starr, G. H. Steam sterilization to kill potato ring rot bacteria on burlap bags. Am. Potato J. **24**, 231-233 (1947).
10. Starr, G. H. and Cinnamon, C. A. Effect of ultra-violet, X-rays and nuclear radiations on ring rot bacteria infesting burlap potato bags. Phytopathology, **43**, 439-440 (1953).
11. Van Schaak, V. Antibiotics and potato ring rot. (Abstract) Phytopathology, **38**, 27 (1948).

CHROMOSOME NUMBERS OF SOME SASKATCHEWAN LEGUMINOSAE WITH PARTICULAR REFERENCE TO ASTRAGALUS AND OXYTROPIS[1]

G. F. LEDINGHAM

Abstract

Chromosome counts have been obtained for 71 collections; seven tribes, 11 genera, and 39 species of the Leguminosae are represented. Pollen mother cells were collected in the field and examined in acetocarmine. Twenty of the species studied were in the tribe Galegeae: *Astragalus* 14 species, *Oxytropis* five species, and *Glycyrrhiza* one species. Twenty-nine of the counts have not been published before. Consideration is given to the phylogeny of *Astragalus*. Evidence suggests that *Astragalus* should be subdivided.

Introduction

Chromosome numbers are known for about 1000 species and 130 genera of Leguminosae (18). This is less than 8% of the 13,000 species and about 25% of the 550 genera. Each tribe and about half of the genera show two or more basic chromosome numbers. It is difficult, therefore, to say what the primary chromosome number in the family is. Although there is no agreement as to how evolution has taken place in the family it is interesting to speculate on the phylogeny of the Leguminosae.

The present study of some Saskatchewan Leguminosae is restricted to plants of the Papilionioideae subfamily, for there are no native Saskatchewan species of either the Mimosoideae or the Caesalpinioideae. Within the Papilionioideae the largest and most controversial genus is *Astragalus*. Since this genus and the closely related *Oxytropis* are well represented in Saskatchewan, special attention was given to the tribe Galegeae in this study.

Taxonomically *Astragalus* is an interesting genus. Its size and variability have led many taxonomists to attempt to subdivide it, using morphological differences. These classifications have not been generally accepted. *Oxytropis* is, however, still recognized as a distinct genus (6, 8) although it can be distinguished from *Astragalus* by only one character, the presence of a sharp projecting point or appendage on the keel.

Cytologically *Astragalus* and *Oxytropis* present a rather confusing picture. Senn (17) reported on 25 Old World species of *Astragalus* which seemed to show a unibasic genus with $x = 8$. There were 19 diploids ($2n = 16$), one tetraploid, two hexaploids, and one octoploid, and two species had $2n = 28$. Since 1938 there have been several studies of North American species of *Astragalus* and they indicate that the genus is multibasic (18). Anderson (1) reported chromosome counts for 10 species of the Galegeae as follows: *Astragalus canadensis*, $2n = 16$; *A. goniatus*, $2n = 16$; *A. striatus*, $2n = 32$; *Oxytropis gracilis*, $2n = 32$; *O. macounii*, $2n = 48$; *Astragalus bisulcatus*

[1]Manuscript received April 30, 1957.
Contribution from Regina College, University of Saskatchewan, Regina, Saskatchewan.

(*Diholcos*), $2n = 24$; *A. pectinatus* (*Cnemidophacos*), $2n = 22$; *A. triphyllus* (*Orophaca*), $2n = 22$; *A. missouriensis* (*Xylophacos*), $2n = 22$; and *A. crassicarpus* (*Geoprumnon*), $2n = 22$. It is interesting to note that species that had been removed from *Astragalus* by Rydberg (16) have $x = 11$ or 12 instead of a number in the 8-series.

Vilkomerson (19) studied the chromosome number relationship of 26 species of *Astragalus*, 16 of which were selenium-absorbers. She found one species with $2n = 16$, 13 with $2n = 22$, one with $2n = 44$, and 11 species with $2n = 24$. James (12) gave chromosome counts for three species, one each of $2n = 22$, 24, and 26. Head (9) reports chromosome counts for 26 species of *Astragalus*; of these one had $2n = 16$, 10 had $2n = 22$, 13 had $2n = 24$, one had $2n = 26$, and one had $2n = 28$. Except for *A. canadensis*, which has $2n = 16$, the plants studied by Head are different species from those studied by Vilkomerson. Turner (18) gives $2n = 26$ as the chromosome number of the one species of *Astragalus* which he studied.

This study reports chromosome counts on 71 collections of 39 different species. These species are in seven tribes and 11 genera. The chromosome numbers of 14 species of *Astragalus* and five species of *Oxytropis* are included in the study. Twenty-nine of the chromosome counts are published for the first time.

Materials and Methods

Cytological material was collected in the field during May, June, July, and August, 1956. All species studied were collected in Saskatchewan and all but two of them are native here. Young inflorescences were collected in Carnoy's 6 : 3 : 1 (absolute alcohol, chloroform, acetic acid). After about one day in the fixative, specimens were usually changed to 70% alcohol. Anthers were squashed in acetocarmine, and the pollen mother cells were examined. If suitable stages were found, the slide was inverted in absolute–acetic (1 : 1) until the cover slip came free, and the slide was then placed in absolute alcohol for a few minutes. The cover slip was then replaced using a drop of diaphane to make a permanent slide.

An attempt was made to collect material from all the Saskatchewan species of the Leguminosae, but several species were not located during the 1956 growing season. The original aim was to investigate the phylogeny of *Astragalus* and *Oxytropis* but other genera were collected so that some comparison of chromosome number and size in the family could be made. Another reason for collecting other genera was because in many cases the chromosome number of Saskatchewan species was unknown. Herbarium specimens were collected and deposited in the herbarium at Regina College, Regina, Saskatchewan.

Observations

Meiotic figures of nearly all pollen mother cell squashes seemed normal. Multivalents, involving four chromosomes, seemed to be common in *Astragalus striatus* and in *Oxytropis sericea* var. *spicata*, attesting to their rather recent

origin as polyploids. One plant of *Hedysarum mackenzii* was found with $2n = 17$. Although the plants studied were collected as widely as possible in Saskatchewan and although four or more counts were made in six different species there was no disagreement between counts.

In the following list of chromosome counts and in the brief notes on the tribes of the subfamily Papilionioideae the arrangement of Gleason (8) is followed. In the genus *Astragalus* the sections of Jones (13) are followed, though several species names are changed to conform with the revision of R. C. Barneby (personal correspondence).

PODALYRIEAE

Thermopsis rhombifolia has $2n = 18$. Anderson (1) made this count in 1950. This agrees with previous counts made in *Thermopsis*. According to Darlington and Wylie (5) *Baptisia*, *Piptanthus*, and *Podalyria* also have $2n = 18$ but five species of *Pultenaea* in Australia differ; one has $2n = 14$, three have $2n = 16$, and one has $2n = 18$ with some triploid and tetraploid plants (4).

GENISTEAE

Lupinus, with two species entering the southwest corner of the province, is the only genus in Saskatchewan. This report of $2n = 48$ for both *L. argenteus* (perennial) and *L. pusillus* (annual) is consistent with previous chromosome counts in the genus. Eleven of 18 species listed by Senn (17) have $2n = 48$.

TRIFOLIEAE

One collection was made in this tribe from the common roadside plant *Melilotus alba*. Much cytological work has been done on the plants of this tribe because of their great economic importance. The basic chromosome number in the tribe is $x = 8$. Of 91 species listed by Senn (17), 69 have $2n = 16$ and 12 have $2n = 32$.

PSORALEAE

The six counts listed here have not been published previously. The numbers reported, $2n = 14$ for three species of *Petalostemum* and $2n = 22$ for three species of *Psoralea*, agree with those reported in 1956 by Turner (18) for eight other species of these two genera. Until Turner's paper was published the basic chromosome number in this tribe seemed to be $x = 10$.

GALEGEAE

The chromosome numbers of 14 species of *Astragalus* are reported. Nine of these are published for the first time. Three of these, *A. missouriensis*, *A. goniatus*, and *A. striatus*, were reported in 1940 by Anderson (1). This study corroborates the work of Vilkomerson (19) by giving the same count for *A. pectinatus* ($2n = 22$), *A. canadensis* ($2n = 16$), *A. bisulcatus* ($2n = 24$), *A. racemosus* ($2n = 24$), and *A. drummondii* ($2n = 22$).

The chromosome counts reported here are of interest because they include first reports in four more sections of Jones's classification of North American

Chromosome numbers of Saskatchewan Papilionioideae

Tribe	Genus and species (section)	Coll. No.*	Location	2n
Podalyrieae	***Thermopsis rhombifolia*** (Nutt.) Richards.	56–1	Lumsden	18
		56–3	Glendenning Park	18
		56–13	Grand Coulee	18
		56–17	Burdick	18
Genisteae	*Lupinus argenteus* Pursh	56–44*b*	Cypress Hills	48
	Lupinus pusillus Pursh	56–43	Big Stick Lake	48
Trifoleae	*Melilotus alba* Desy. (escape)	56–100	Regina, 20 N.	16
Psoraleae	*Petalostemum candidum* (Willd.) Michx.	56–105	Moosomin	14
	Petalostemum purpureum (Vent.) Rydb.	56–93	Regina, 20 N.	14
	Petalostemum villosum Nutt.	56–121	Mortlach	14
	Psoralea argophylla Pursh	56–96	Regina, 22 N.	22
	Psoralea esculenta Pursh	56–89	Cypress Lake	22
	Psoralea lanceolata Pursh	56–85	Pike Lake	22
Galegeae	(Homalobi)			
	Astragalus tenellus Pursh	56–55	Regina, 22 N.	24
	Astragalus spatulatus Sheld.	56–40	Cypress Lake	24
	(Triphylli)			
	Astragalus gilviflorus Sheld.	56–9	Rouleau	24
	(Alpini)			
	Astragalus eucosmus Robins.	56–26	Saskatoon (cult)	32
	Astragalus aboriginum Richards.	56–86	Borden	16
	(Podo-sclerocarpi)			
	Astragalus pectinatus Dougl.	56–38	W. Cypress Hills	22
		56–42	Piapot, 12 N.	22
	(Uliginosi)			
	Astragalus canadensis L.	56–101	Regina, 18 N.	16
		56–116	Gainsborough	16
	(Hypoglottides)			
	Astragalus striatus Nutt.	56–70	Estevan, 6 SE.	32
		56–88	Borden	32
	Astragalus goniatus Nutt.	56–21	Cactus Hills	16
		56–71	Estevan	16
		56–72	Estevan	16
	(Flexuosi)			
	Astragalus flexuosus Dougl.	56–52	Regina, 22 N.	22
		56–53	Regina, 22 N.	22
		56–78	Duval	22
		56–104	Moosomin, 8 S.	22
	(Argophylli)			
	Astragalus missouriensis Nutt.	56–12	Avonlea	22
		56–15	Craven	22
	(Bisulcati)			
	Astragalus bisulcatus (Hook.) Gray	56–47	Cypress Lake	24
		56–83	Moon Lake	24
	(Galegiformes)			
	Astragalus drummondii Hook.	56–46	Cypress Lake	22
	Astragalus racemosus Pursh	56–120	Moose Jaw	24
	Oxytropis deflexa (Pall.) DC.	56–79	Moon Lake	16
	Oxytropis splendens Dougl.	56–97	Regina, 22 N.	16
		56–98	Regina, 22 N.	16
		56–114	Carievale	16
		56–118	Regina, 18 NW.	16
	Oxytropis campestris (L.) DC. var. *gracilis* (A.Nels.) Barneby	56–18	Burdick	32
		56–22	Crestwynd	32
		56–25	Regina, 5 NE.	32
		56–50	Cypress Lake	32
		56–82	Moon Lake	32
		56–107	Moosomin, 6 S.	32
	Oxytropis lambertii Pursh	56–69	Estevan	48
	Oxytropis sericea Nutt. var. *spicata* (Hook.) Barneby	56–5	Lumsden	48
		56–10	Rouleau	48
		56–19	Burdick	48
		56–23	Crestwynd	48
		56–32	Cypress Hills	48
	Glycyrrhiza lepidota Pursh	56–58	Regina, 22 N.	16
		56–65	Macoun, 7 S.	16
		56–96	Moose Jaw (cult)	16
		56–110	Moosomin	16
Hedysareae	***Hedysarum alpinum*** **L. var.** ***americanum*** **Michx.**	56–59	Regina, 20 N.	14
		56–91	Regina, 12 E.	14
		56–113	Redvers, 10 N.	14

CHROMOSOME NUMBERS OF SASKATCHEWAN PAPILIONIOIDEAE—*Concluded*

Tribe	Genus and species (section)	Coll. No.*	Location	$2n$
	Hedysarum boreale Nutt. var. *cinerascens* (Rydb.) Rollins	56–73	Roche Percee	16
		56–75	Regina, 22 N.	16
	Hedysarum mackenzii Richards.	56–90	Langham	16
		1278	Langham (1952)	17
Vicieae	*Lathyrus ochroleucus* Hook.	56–27	Regina	14
	Lathyrus venosus Muhl.	1301	Saskatoon (1952)	28
	Vicia americana Muhl.	56–57	Regina, 22 N.	14
	Vicia cracca L. (introduced)	56–117	Carnduff	28
	Vicia sparsifolia Nutt.	56–11	Avonlea	14
	Vicia trifida Dietr.	56–20	Cactus Hills	14

*Cytological collections were made from May 14 to July 22, 1956, and were numbered consecutively from 56–1 to 56–121. Data are included from two collections made in 1952.

species of *Astragalus* (13). Two of these sections, Triphylli and Flexuosi, have $x = 12$ and $x = 11$ respectively. The other two sections, Alpini and Hypoglottides, are based on an 8-chromosome series, and the species studied include one diploid and one tetraploid in each section.

The five species of *Oxytropis* studied include two diploids, one tetraploid, and two hexaploids. They seem to be a closely related group based on an 8-chromosome series. Multivalents are common, especially in *O. sericea* var. *spicata*; four tetravalents and one hexavalent are shown in Fig. 28. The most conspicuous difference between this taxon and *O. campestris* var. *gracilis* is the size of the flower and the fruit. These two species were studied somewhat more intensively than others because of some doubt about their distinctness. Both are widespread and common in southern Saskatchewan. *O. sericea* var. *spicata* flowers early but there is an overlapping of its flowering dates with those of *O. campestris* var. *gracilis*, as is shown by the collection numbers. On May 24, 1956, these two species were collected (56–18 and 56–19) in the same area at Burdick, 8 miles east of Moose Jaw, and again (56–22 and 56–23) near Crestwynd, 28 miles south southwest of Moose Jaw. Crossing could take place in the field but no cytological evidence of hybridization or intergradation between these two species was found. The chromosome number $2n = 36$ was reported for Scandinavian *O. campestris* by Jalas in the chromosome list by Löve and Löve (14) and this number was repeated by Darlington and Wylie (5). After a closer examination of more material, Jalas (11), however, corrected this number as a mistake, since he had counted $2n =$ ca. 48 chromosomes in the typical ssp. *campestris*, originally described from this region, as well as in ssp. *sordida* (Willd.) Hartm. Since the Saskatchewan *O. gracilis* (A. Nels.) K. Schum. has been found to have $2n = 32$ chromosomes only, this must be taken as a strong indication that it is a good species and not a variety only of *O. campestris* as proposed by Barneby (2).

Glycyrrhiza lepidota has $2n = 16$. The chromosome number of this species was reported in 1948 (10). Five Old World species of *Glycyrrhiza* also have $2n = 16$ (5).

HEDYSAREAE

In this tribe the basic chromosome numbers 7, 8, 9, 10, and 11 are found (17). Only *Hedysarum* occurs in Saskatchewan. This genus of about 90 species occurs mainly in the north temperate and subarctic zones of Eurasia with only six species in North America (8). Anderson (1) gives the same counts $2n = 14$ for *H. alpinum* var. *americanum* and $2n = 16$ for *H. boreale* var. *cinerascens* and *H. mackenzii* as are here reported. Four Old World species have been reported, three have $2n = 14$ and one has $2n = 16$ (5). One plant of *H. mackenzii* in this study was found to have $2n = 17$.

VICIEAE

The basic chromosome number in this tribe is 7. Of 37 species of *Vicia* listed by Senn (17), 22 have the diploid number $2n = 14$ and seven are polynumeral, i.e. different strains within the species have different chromosome numbers. It is interesting to note that of the four strains of *V. cracca*, $n = 6$, 7, 12, and 14, it is the highest chromosome number, $2n = 28$, which has become established in Saskatchewan. The other three species of *Vicia* listed here are closely related to each other and may be considered as varieties of *V. americana* Muhl. *Lathyrus* has an even more stable chromosome number. Of 42 species listed (17), 41 have $2n = 14$. Senn (17) gives the first report of *Lathryus venosus*, $2n = 28$.

Discussion

Outside the tribe Galegeae this paper lists 19 species of the Leguminosae. Chromosome counts for 16 of these are reported for the first time. In each case previous counts have been made in the genus and counts reported here are in agreement with published reports on related species. There is little taxonomic disagreement about the species or genera of these tribes but more information might show the necessity of redefining and separating some of the tribes.

FIGS. 1–33. Meiotic chromosomes of species of Leguminosae.

FIG. 1. *Thermopsis rhombifolia* ($n = 9$). FIG. 2. *Lupinus argenteus* ($n = 24$). FIG. 3. *Lupinus pusillus* late prophase of first division ($n = 24$). FIG. 4. *Melilotus alba* ($n = 8$). FIG. 5. *Petalostemum candidum* ($n = 7$). FIG. 6. *Petalostemum villosum* ($n = 7$). FIG. 7. *Psoralea argophylla* ($n = 11$). FIG. 8. *Psoralea esculenta* ($n = 11$). FIG. 9. *Psoralea lanceolata* ($n = 11$). FIG. 10. *Astragalus tenellus* ($n = 12$). FIG. 11. *Astragalus spatulatus* ($n = 12$). FIG. 12. *Astragalus gilviflorus*, anaphase of first division ($n = 12$). FIG. 13. *Astragalus eucosmus* ($n = 16$). FIG. 14. *Astragalus aboriginum* ($n = 8$). FIG. 15. *Astragalus pectinatus* ($n = 11$). FIG. 16. *Astragalus canadensis* ($n = 8$). FIG. 17. *Astragalus striatus* $12_{II} + 2_{IV}$ ($n = 16$). FIG. 18. *Astragalus goniatus* ($n = 8$). FIG. 19. *Astragalus flexuosus* ($n = 11$). FIG. 20. *Astragalus missouriensis* ($n = 11$). FIG. 21. *Astragalus bisulcatus* ($n = 12$). FIG. 22. *Astragalus drummondii*, anaphase of first division ($n = 11$). FIG. 23. *Astragalus racemosus* ($n = 12$). FIG. 24. *Oxytropis deflexa* ($n = 8$). FIG. 25. *Oxytropis splendens* ($n = 8$). FIG. 26. *Oxytropis campestris* var. *gracilis* ($n = 16$). FIG. 27. *Oxytropis lambertii* ($n = 24$). FIG. 28. *Oxytropis sericea* var. *spicata* $13_{II} + 4_{IV} + 1_{VI}$ ($n = 24$). FIG. 29. *Glycyrrhiza lepidota* ($n = 8$). FIG. 30. *Hedysarum alpinum* var. *americanum* ($n = 7$). FIG. 31. *Hedysarum boreale* var. *cinerascens* ($n = 8$). FIG. 32. *Hedysarum mackenzii* ($n = 8$). FIG. 33. *Lathyrus ochroleucus* ($n = 7$).

All figures approximately × 875.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33

The most interesting counts are those obtained for *Petalostemum* and *Psoralea* of the tribe Psoraleae. The counts reported here, $n = 7$ and $n = 11$, agree with those given by Turner (18). Previous counts in this tribe, even including some species of *Psoralea*, indicated a basic chromosome number of $x = 10$ (17).

In the Galegeae, 20 species have been studied and 13 chromosome counts are published for the first time. Old World species of *Astragalus* seem to be based entirely on an 8-chromosome series (17). Counts made on North American species suggest that *Astragalus* is a multibasic genus (1, 9, 12, 18, 19). This study supports the idea that there are at least two basic chromosome numbers in *Astragalus*. Saskatchewan species fall into two groups. One group, including nine of the 14 species of *Astragalus* studied, has $2n = 22$ or $2n = 24$. The second group, containing only five species, has $2n = 16$ or $2n = 32$. The first group, with the larger basic chromosome number, seems to be the characteristic American group. A total of 63 North American species of *Astragalus* have been counted; 58 of them fit into this group. The second group, based on the 8-series, has fewer species in North America. Species of the 8-series are native to northern North America, while species of the other, larger group are generally more southern in their range. For this reason Saskatchewan, where species of both groups are to be found, is an especially favorable *Astragalus* study area.

The five North American 8-chromosome *Astragalus* species are all plants of the Middle and Upper Temperate Life Zones and they have close relatives or varieties in Asia and Europe (13). Since these northern species have chromosome numbers identical with those of the Old World species of *Astragalus* (17), their origin in North America would seem to be by migration from Siberia during the Pleistocene. Two of these five species are tetraploid.

The species of the characteristic American group have the basic chromosome number $x = 11$ or 12, and a few have $x = 13$ or 14. Of 58 now known only *Astragalus grayi* in section Podo-sclerocarpi is polyploid with $2n = 44$ (19). The scarcity of polyploidy in the group suggests that it has little familiarity with cold northern conditions (15). Since it is richer in species than the northern group, it has probably been in America for a much longer time. Members of this group could have migrated into North America through the tropics from the south where Croizat (3) says that all primary angiospermous groups originated. The three South American species of *Astragalus* so far studied all have $2n = 26$. Typical American species of *Astragalus*, therefore, seem to have been separated from Eurasian and from American 8-chromosome species for a long time.

The cytological evidence strongly suggests that there are two branches or two lines of evolution in the genus *Astragalus*. This is not clearly supported by morphological evidence. Perhaps a more complete study of the anatomy, development, or biochemistry of the species of *Astragalus* will show other basic differences between the species of the two branches of the genus. Even without other supporting evidence it should be recognized that *Astragalus* is composed of at least two important subgenera.

Oxytropis contains about 200 species of circumboreal distribution with about 40 species in North America (8). The species studied resemble the 8-chromosome species of *Astragalus* by having similar chromosome numbers, by their polyploidy, and by their distribution and probable migration into North America from the northwest. Cytologically, it is obvious that *Oxytropis* is more closely related to the 8-chromosome group of *Astragalus* species than the two groups of *Astragalus* are related to each other.

Barneby (2) describes hybrids between certain species of *Oxytropis*. Since both *O. lambertii* and *O. sericea* are hexaploid it is evident that hybridization and polyploidy have been important in the evolution of *Oxytropis*. It is interesting to note that tetravalents were absent in the cells of *O. lambertii* studied, but each first metaphase cell in *O. sericea* had three or four multivalents, sometimes more, as shown in Fig. 28. This may indicate that *O. sericea* is of more recent origin than *O. lambertii* (7).

Chromosomes of *Glycyrrhiza lepidota* (Fig. 29) are smaller than the chromosomes of $x = 8$ species of *Astragalus* (Figs. 13, 14, 16, 17, 18) and of species of *Oxytropis* (Figs. 24, 25, 26, 27, 28). The distribution of the genus also indicates that *Glycyrrhiza* is not closely related to either *Astragalus* or *Oxytropis*.

Although polyploidy occurs, it does not appear to have been a common method of chromosome number change in the Leguminosae. Aneuploidy is, however, very common within the family. Each tribe of the family has two or more basic chromosome numbers, usually in a continuous series. This tendency is shown also by at least half of the genera in which more than one species has been studied. Number change is even shown in some species, e.g. species of *Vicia* (5). An important method of evolution in the Leguminosae, therefore, has been the occurrence of changes which led to increase or decrease of chromosome number. *Hedysarum* has $x = 7$ and $x = 8$. *Astragalus* is the only genus in which different species show a conspicuous difference in chromosome numbers. There can have been no gene exchange between members of the two main groups of *Astragalus* for a long time. It seems likely, therefore, that the American species of *Astragalus* with $x = 11$, 12, 13, 14 should eventually be separated into a different genus from the 8-chromosome Old World species of *Astragalus*.

Acknowledgments

The writer is indebted to R. C. Barneby, Wappingers Falls, New York, for checking the identifications of *Astragalus* and *Oxytropis* species; to T. J. Arnason, Biology Department, University of Saskatchewan, and to A. Löve, Institut Botanique, University of Montreal, for reading this paper and giving helpful suggestions; and to Miss Suzanne Yip, Saskatoon, for technical assistance throughout the project. Financial aid was provided by the National Research Council of Canada in the form of a grant (P.R. 114).

References

1. ANDERSON, M. M. Chromosome numbers of Saskatchewan legumes. Master's Thesis, University of Saskatchewan, Saskatoon, Saskatchewan. 1940.
2. BARNEBY, R. C. A revision of the North American species of *Oxytropis* DC. Proceedings of the California Academy of Sciences, **27**, 177–312 (1952).
3. CROIZAT, L. Manual of phytogeography. Junk, The Hague. 1952.
4. CURTIS, W. M. Variation in certain Tasmanian plants. New Phytologist, **51**, 398–414 (1952).
5. DARLINGTON, C. D. and WYLIE, A. P. Chromosome atlas of the flowering plants. Macmillan Publishing Company, New York, N.Y. 1955.
6. FERNALD, M. L. Gray's Manual of botany. 8th ed. American Book Company, New York, N.Y. 1950.
7. GILLES, A. and RANDOLPH, L. F. Reduction of quadrivalent frequency in autotetraploid maize during a period of 10 years. Am. J. Botany, **38**, 12–17 (1951).
8. GLEASON, H. A. The new Britton and Brown illustrated flora of the Northeastern United States and adjacent Canada. N.Y. Botan. Garden, New York. 1952.
9. HEAD, S. C. Mitotic chromosome studies in the genus *Astragalus*. Master's Thesis, State College, Pullman, Washington. 1955.
10. HEISER, C. B., JR. and WHITAKER, T. W. Chromosome number, polyploidy, and growth habit in California weeds. Am. J. Botany, **35**, 179–186 (1948).
11. JALAS, J. Zur Kausalanalyse der Verbreitung einiger nordischen Os- und Sandpflanzen. Ann. Bot. Soc. Zool. Bot. Fennica Vanamo, 24, **1**, 1–362 (1950).
12. JAMES, L. E. Observations on the taxonomy of *Astragalus*, subgenus *Hesperastragalus*. Contrib. Dudley Herbarium, **4**, 63–72 (1951).
13. JONES, M. E. Revision of North American species of *Astragalus*. Salt Lake City, Utah. 1923.
14. LÖVE, A. and LÖVE D. Chromosome numbers of Northern plant species. Iceland University Inst. Appl. Sci. Dep. Agr. Rept. B, **3**, 1–131 (1948).
15. LÖVE, A. and LÖVE D. The geobotanical significance of polyploidy. 1. Polyploidy and latitude. Portugaliae Acta Biol. Sér. A. 273–352 (1949).
16. RYDBERG, P. A. Flora of the prairies and plains of Central North America. N.Y. Botan. Garden, New York. 1932.
17. SENN, A. H. Chromosome number relationship in the Leguminosae. Bibliographia Genet. **12**, 175–336 (1938).
18. TURNER, B. L. Chromosome numbers in the Leguminosae. Am. J. Botany, **43**, 577–581 (1956).
19. Vilkomerson, H. Chromosomes of *Astragalus*. Bull. Torrey Botan. Club, **70**, 430–436 (1943).

THE LOWBUSH BLUEBERRY: THE VASCULAR ANATOMY OF THE OVARY[1]

HUGH P. BELL AND ELSPETH C. GIFFIN

Abstract

The vascular anatomy of the blueberry flower ovary is described in detail. Ten primary flower bundles branch off from a siphonostele in the pedicel. From these are formed the sepal, petal, stamen, dorsal carpellary, ventral carpellary, and nectary bundles. The levels recorded for the branching of certain bundles are not in agreement with previous accounts. The possible reasons for this apparent disagreement are discussed. All structures are illustrated by 11 mechanically constructed diagrams.

Introduction

The vascular anatomy of the ovary of *Vaccinium pennsylvanicum* has been described by Hancy (7) but her article is an M.A. thesis in the library of Cornell University and has never been published; hence it is not readily or widely accessible, but a number of investigators must have needed the information it contains for Miss Hancy has been quoted by many including Baker (1), Palser (9 and 10), Chou (3), Kavaljian (8), and Eames and MacDaniels (5). Her descriptions are very brief, and in a number of details they do not agree with the observations made during the present investigation. With all these facts in mind, it appeared desirable to publish the description given below.

Materials and Methods

The species described is *Vaccinium angustifolium* var. *laevifolium*. The tracing of the vascular anatomy was done from a continuous series of transverse sections. Critical sections were plotted mechanically on squared graph paper. Each millimeter on this paper represented a constant number of microns. Vertical distances were calculated by counting the number of sections each 8 microns in thickness. Horizontal distances were measured by a calibrated micrometer eyepiece. Then the longitudinal section was constructed from the transverse diagrams. This longitudinal diagram was checked against a number of continuous longitudinal series through ovaries collected on the same date. The constructed longitudinal diagram and the actual longitudinal sections agreed in every detail. The series used were through the ovaries of flowers at the time of full bloom, but these were checked with younger ovaries and with older ovaries to the ripe fruit stage, and although there was naturally great change in size and maturity, there was no change in the vascular pattern. For many reasons the ovary at the time of full bloom provided material which could be most accurately interpreted. That is why ovaries from flowers at this stage were used.

[1]Manuscript received May 30, 1957.
Contribution from the Nova Scotia Research Foundation, Halifax, Nova Scotia.

Descriptions of Structure

The Base of the Ovary

In the pedicel, the vascular system is a siphonostele (Fig. 1). At the base of the ovary this breaks into 10 branches which pass diagonally outward and upward. These are the primaries from which the whole vascular system originates. Five of these primaries branch off from the pedicel slightly lower (Fig. 2) than the five that alternate with them (Fig. 3). The lower five are the primaries from which the petal, dorsal carpellary, and petal–stamen bundles will arise, and the five alternate primaries will give rise to the sepal and the sepal–stamen bundles. However, from each of these primaries, before they reach their ultimate peripheral position, there is given off a branch which extends towards the center (Fig. 11). Each branch originates by a periclinal or tangential division of a primary bundle. In all cases of such periclinal branching of bundles, the phloem strands may be followed around the outside of the xylem to their position in the branch. Each of these first branches from the primaries forms an acute angle with the lower and more centrally located part of the primary. As 10 bundles they extend into the center of the ovary and from them the ventral carpellary system originates.

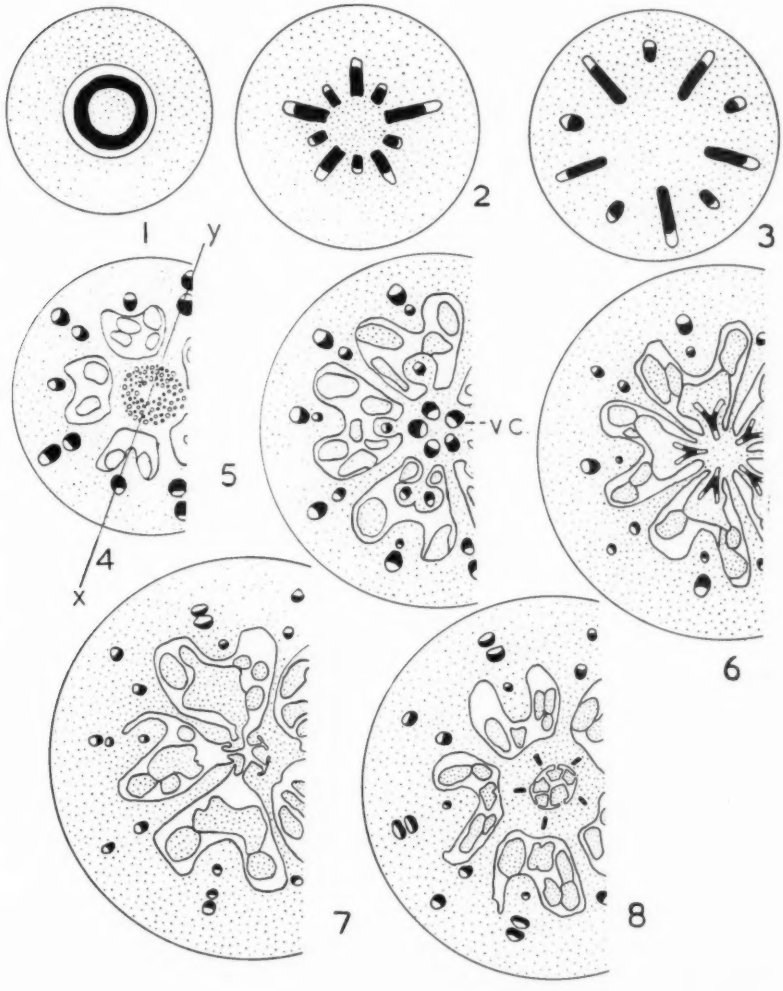
The Sepal and Sepal–Stamen System

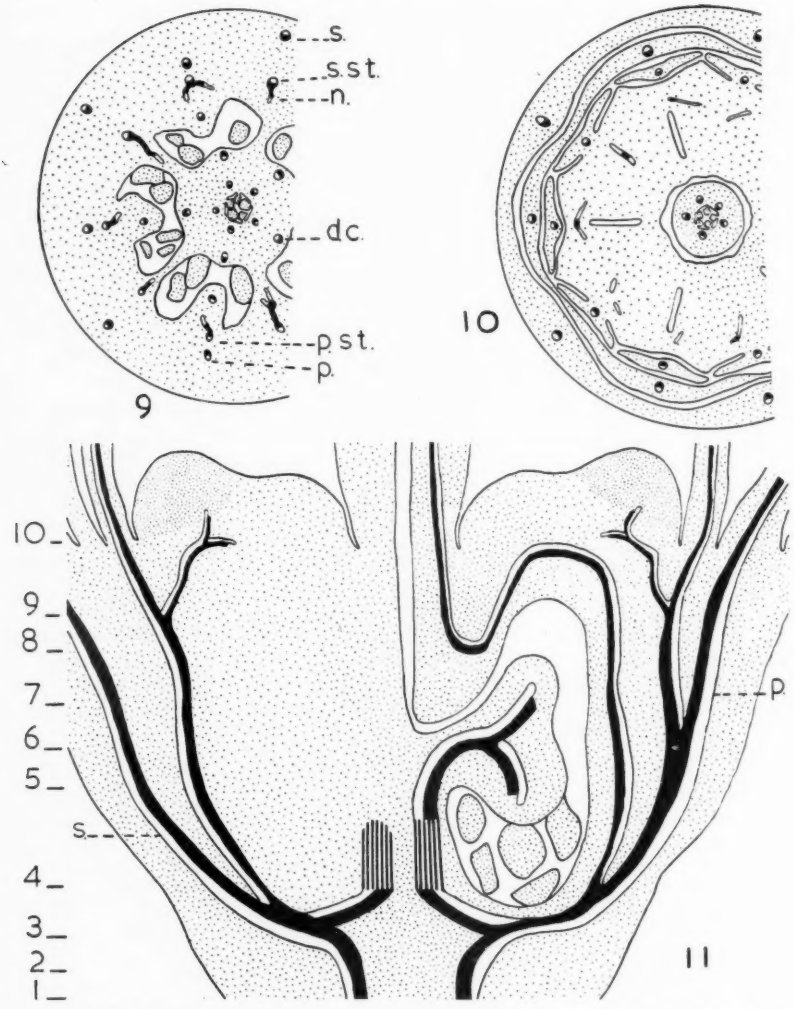
At a very short distance from the ventral carpellary branch, each of the five primaries that originate at the slightly higher level branches again. This branching occurs below the base of the loculi and it is again a periclinal division. The inner branch resulting from this bifurcation is somewhat the smaller and extends upward through the flesh of the ovary until it enters the base of a stamen. This stamen is one of the inner circle of stamens. The outer branch also extends upward through the flesh and enters the base of a sepal of which it forms the mid-rib and largest sepal bundle. Both the sepal bundle and the sepal–stamen bundle are opposite the partition between the loculi, and in the center of the ovary the stamen bundle lies

---

In all diagrams the xylem is solid black, the phloem white, and the flesh stippled. FIGS. 1–8 are mechanically constructed diagrams of transverse sections at successively higher levels, × 25. FIG. 1 is taken as level zero. For each of the other diagrams the distance of the section above the level of FIG. 1 is given in microns. All diagrams are oriented so that a lower diverged primary bundle or the center of a loculus or a dorsal carpellary bundle or a petal bundle is at 9 o'clock. FIG. 1 (Level 0). The vascular tissue is a siphonostele. FIG. 2 (Level 90). The lower diverged primaries are differentiating horizontally outward. FIG. 3 (Level 195). The higher diverged primaries are differentiating horizontally outward. FIG. 4 (Level 350). The sepal–stamen bundles are diverged. The ventral carpellary tissue is not yet organized into bundles: *x–y*, plane of FIG. 11. FIG. 5 (Level 652). The dorsal carpellaries are diverged. The ventral carpellary vascular tissue is organized into five bundles with the phloem on the inside: *v.c.*, ventral carpellary bundle. FIG. 6 (Level 778). The ventral carpellaries are extended horizontally into the placentae. FIG. 7 (Level 912). The petal–stamen bundles are diverged or are diverging. This section is cut at a slightly oblique angle, hence it includes a placenta partially attached, a partition the inner end of which is free and at the right, partitions the inner portions of which are united at the base of the style. FIG. 8 (Level 1074). The lower limit of the inner and lower dorsal carpellary loop is seen as a horizontal strand of xylem.

just slightly inside the partition. Both the sepal bundle and the stamen bundle throughout their whole length give off numerous fine anastomosing branches. Most of the branches from the sepal bundle extend outward and supply the outer flesh of the ovary. The branches from the stamen bundle mostly extend inward but some branches from each may go either way. These fine branches are so indefinite and irregular that they are not shown in the accompanying diagrams. Some of these fine branches extend to the top of the ovary and enter the base of the sepal. As a result there are a





In all diagrams the xylem is solid black, the phloem white, and the flesh stippled. FIGS. 9, 10 mechanically constructed diagrams of transverse sections, × 25. For each diagram, the distance of the section above the level of FIG. 1 is given in microns. The diagrams are oriented with a petal bundle at 9 o'clock. FIG. 9 (Level 1181). There are three transverse sections of each dorsal carpellary. In the center view, the phloem is on the inside. Nectary bundles are diverging horizontally inward: *d.c.*, dorsal carpellary bundle; *n.*, nectary bundle; *p.*, petal bundle; *p.st.*, petal–stamen bundle; *s.*, sepal bundle; *s.st.*, sepal–stamen bundle. FIG. 10 (Level 1409). The upper limit of the outer and upper dorsal carpellary loop is seen as a horizontal strand of phloem. There are numerous scattered branching strands of nectary bundles. FIG. 11. A mechanically constructed longitudinal diagram of ovary through plane *x–y*, FIG. 4, × 50. The level of each transverse diagram is indicated on the left. The nectary is more deeply stippled: *p.*, petal bundle; *s.*, sepal bundle.

varying number of small bundles in each sepal, but although these vary greatly in size they are never as large as the mid-rib, and sometimes they are so small that the xylem may contain only a couple of tracheids. Near the top of the ovary, there is a small but conspicuous branch from the stamen bundle. This originates by periclinal division and proceeds inward just below the nectary. It is tortuous in its course and ramifies repeatedly. When it reaches the base of the nectary the xylem elements end but phloem elements continue for a short distance as branching strands between the lower nectary cells.

The Petal, Petal–Stamen, and Dorsal Carpellary System

The five primaries which arise at the slightly lower level do not branch a second time as close to the center of ovary as the other five do. From these five lower primaries a second and inner branch comes off at about the level of the base of the loculus and at a distance from the center just below the inner limit of the false partition. This second branch given off from this primary is the dorsal carpellary bundle. It originates usually by an oblique anticlinal or radial division from one side of the primary, hence it does not always lie on the same radius as the primary from which it branches, and higher in the ovary it may not be on the same radius as the petal and petal–stamen bundles. This condition was noted by Baker (1) for *Vaccinium macrocarpon*. This mode of origin is not absolutely constant for in a few cases it may vary all the way from true periclinal to true anticlinal. This dorsal carpellary extends upward through the false partition, curves over the top of the loculus, down through the flesh on the inside of the loculus, again turns toward the center, enters the base of the style, bends upward until it becomes vertical, and finally proceeds upward in the style. That is, this bundle twice changes direction through 180 degrees and in doing so forms two complete loops. In the ovary used for these transverse diagrams, the distance from the bottom of the lower and inner loop (Fig. 8) to the top of the higher and outer loop (Fig. 10) was 335μ. It is obvious that in a cross section through the mid upper half of the loculus (Fig. 9) a cross section of the dorsal carpellary will be seen in three places, and in the central one of these cross sections the phloem will be on the inside. After the dorsal carpellary branches off, the outer and larger portion of the primary continues upward as a single strand to about the level of the center of the loculus where it branches again (Fig. 7). This time it branches by a true periclinal division. The inner and smaller of the two branches continues upward and finally enters one of the stamens of the outer ring. Just below the base of the nectary a nectary branch comes off from this stamen bundle. It is exactly similar to the nectary branch already described as coming off from the sepal–stamen bundle. Thus there is a total of 10 nectary branches. The outer and larger branch of the primary is the petal bundle. It continues as a single strand into the petal. The petal–stamen bundle gives off many fine branches similar to those given off from the sepal and sepal–stamen bundles. The branches from the petal–stamen bundle mostly proceed inward. No branches

were observed from either the petal bundle or the dorsal carpellary bundle. It is obvious that this whole petal, petal–stamen, dorsal carpellary system lies in the ovary wall opposite the dorsal center of the loculus.

The Ventral Carpellaries

As stated above, the ventral carpellary system originates near the base of the ovary as 10 inward extending branches from the primaries. These meet in the center of the ovary at the base of the central column. There they at first form an irregular vascular ring in which the phloem and xylem are completely mixed without any definite arrangement (Fig. 4). The lack of arrangement persists for a short distance upwards but soon this vascular tissue becomes organized into five bundles (Fig. 5). Each of these bundles is opposite a loculus and the phloem is inside the xylem. These continue upward as five distinct bundles to the level of the placentae where they bend outward and enter the placentae (Fig. 6). In the placentae they branch repeatedly, one branch going to each ovule.

Discussion

The account given above differs from previous accounts as follows. Miss Hancy (7) describes the 10 primaries as coming off from the pedicel at the same level. In the present investigation it was observed that five came off at a slightly lower level than the five that alternated with them. Referring to the vascular cylinder of the pedicel Miss Hancy (7) says this mass breaks immediately into 10 outer and 10 inner bundles. In the work reported above, the 10 bundles that will form the ventral carpellary system were observed as 10 inward extending branches from the already laterally extended primaries. Miss Hancy (7) does not mention the branches to the nectaries or the reversal of position of the xylem and phloem in the ventral carpellaries. Eames (4) in his Figure 30*g* illustrates *Vaccinium pennsylvanicum*. In his figure the dorsal carpellary and the petal–stamen bundles branch off at a much higher level than the level given in the present article. There are two possible explanations for this apparent disagreement: one, the species may vary from region to region, or two, although Fernald (6) and other authors give *V. pennsylvanicum* as a synonym of *V. angustifolium* var. *laevifolium*, Camp (2) suggests that there is some doubt as to the species indicated when the name *V. pennsylvanicum* was used during the earlier half of this century; hence the plants examined by both Miss Hancy and Dr. Eames may not have been the species common in Nova Scotia and now called *V. angustifolium* var. *laevifolium*, for it is very unlikely that Dr. Eames would be in error in such a matter.

Acknowledgments

All the figures in this article were drawn by the junior author. Again the authors wish to express their indebtedness to Dalhousie University where laboratory space was provided for the work.

References

1. BAKER, D. G. The development and vascular anatomy of the flower of *Vaccinium macrocarpon*. Thesis, University of Chicago, Chicago, Illinois. 1932.
2. CAMP, W. H. Another new name in *Vaccinium*. Bull. Torrey Botan. Club, **71**, 179–180 (1944).
3. CHOU, Y.-L. Floral morphology of three species of *Gaultheria*. Botan. Gaz. **114**, 198–221 (1952).
4. EAMES, A. J. The vascular anatomy of the flower with refutation of the theory of carpel polymorphism. Am. J. Botany, **18**, 147-188 (1931).
5. EAMES, A. J. and MACDANIELS, L. H. An introduction to plant anatomy. 2nd ed. McGraw-Hill Book Co., Inc., New York. 1947.
6. FERNALD, M. S. Gray's Manual of botany, 8th ed. American Book Co., New York. 1950.
7. HANCY, A. J. The vascular anatomy of certain ericaceous flowers. Thesis, Cornell University, Ithaca, N.Y. 1916.
8. KAVALJIAN, L. G. The floral morphology of *Clethra alnifolia* with some notes on *C. acuminata* and *C. arborea*. Botan. Gaz. **113**, 392–413 (1952).
9. PALSER, B. F. Studies of floral morphology in the Ericales. I. Organography and vascular anatomy in the Andromedeae. Botan. Gaz. **112**, 447–485 (1951).
10. PALSER, B. F. Studies of floral morphology in the Ericales. III. Organography and vascular anatomy in several species of the Arbuteae. Phytomorphology, **4**, 335–354 (1954).

DEFINITIONS OF TERMS IN FOREST AND SHADE TREE DISEASES[1]

Compiled by D. C. BUCKLAND,[2] D. R. REDMOND,[3] AND R. POMERLEAU[4]

In 1948, the Canadian Phytopathological Society established a committee to study terminology in plant pathology. This committee, after evaluating the needs, decided to proceed with attempts to improve or standardize terminology in specific fields of plant pathology: shade tree pathology, forest pathology, and virus pathology. Therefore, subcommittees were formed to initiate terminology surveys in these fields, and to prepare completed reviews for publications.

The review of terms used in forest pathology is the first of these completed. The senior author, who served as subcommittee chairman, prepared a list of terms and definitions, solicited the comments of other forest pathologists in Canada, and incorporated their suggestions. The junior authors assisted in the final phases of the list and in preparing terms specific for shade tree pathology, respectively.

Considerable use was made of definitions for forest disease terms included in two previously published reviews in the broad field of forest terminology (1, 2). Some of the definitions in this review are adopted from one or both of the earlier reviews; others are revisions of definitions in these publications. Many terms commonly used in phytopathology but not specific to forest pathology have been omitted.

Terminology, being arbitrary, must be based on sound concepts of a majority of workers in a field, and can seldom command universal acceptance. It must always remain subject to revision and improvement. There is no expectation or desire that this review will be accepted without question by all workers. The review has been prepared as an attempt to provide terminology specifically adapted to the needs of forest pathologists. The sponsoring societies hope that, if there are important omissions or misconceptions in this presentation, other groups of workers will proceed to correct them in future by means of similar terminology reviews. Until such revisions appear they hope that this review will enjoy general acceptance, and will encourage standardization of terminology in forest pathology.

References

1. EMPIRE FORESTRY ASSOCIATION. British Commonwealth forest terminology Part I. Royal Empire Society. London. 1953.
2. SOCIETY OF AMERICAN FORESTERS. Forestry terminology. Washington, D.C. 1950.

[1]Manuscript received May 27, 1957.
Prepared under joint sponsorship of the Canadian Phytopathological Society and the Canadian Institute of Forestry.
[2]Associate Professor of Forest Pathology, University of British Columbia, Vancouver, British Columbia. (Deceased.)
[3]Forest Biologist, Canada Department of Agriculture, Forest Biology Laboratory, Fredericton, New Brunswick.
[4]Forest Biologist, Canada Department of Agriculture, Forest Biology Laboratory, Quebec, Quebec.

FOREST PATHOLOGY TERMS

1. Age-decline—Age of a tree at which damage by biotic factors equals annual increment.
2. Age, infection—Age of host at time of infection.
3. Age, pathological cutting—A cutting age determined by pathological factors.
4. Area, ribes-free—In blister rust control, an area free of *Ribes* plants, or almost so.
5. Black-heart—See Stain, mineral.
6. Black-line—See Line, zone.
7. Blight, parch—See Winter drying.
8. Blight, snow—A disease of coniferous foliage and twigs, caused by *Phacidium* spp. that develop under snow.
9. Canker, perennial—A canker in which intermittent activity of the pathogen or other injurious factor over a period of years prevents complete healing.
10. Canker, target—A perennial canker characterized by prominent, concentrically arranged zones, which mark successive outgrowth and death of tissues surrounding the wound.
11. Cast, leaf—A premature shedding of leaves.
12. Cast, needle—Leaf cast of needle-bearing conifers.
13. Cedar-apple—A gall caused by *Gymnosporangium* spp. on *Juniperus* spp.
14. Conk—The fruit-body of a wood destroying fungus, especially of a polypore.
15. Conk, blind—An overgrown decayed knot; the overgrowth usually abnormally protruded.
16. Conky—Applied to a log or tree bearing sporophores of wood-rotting fungi.
17. Crack, frost—A longitudinal radial split in the trunk of a tree, generally near the base, caused by stresses due to low temperatures.
18. Cull—(1) An inferior plant rejected from nursery stock.
    (2) A tree or log rejected because of defect.
    (3) Any log or timber so defected.
    (4) The lowest grade of lumber. Also used in a verbal sense.
19. Cull, commercial—The volume of material unmerchantable under prevailing utilization practice.
20. Cull, mill—Losses in milling due to sweep, rot, shake, or other defect.
21. Cull, per cent—The ratio of unmerchantable to total wood volume of commercial species.
22. Cull, theoretical—Cull based on theoretical considerations and not on actual practice.
23. Cup, shot-hole—A hollow or depression in the bark with worm holes, which indicates the former site of *Polyporus amarus* on *Libocedrus decurrens*.
24. Cutting, salvage—The cutting and removal of trees killed or injured badly to utilize merchantable material before it becomes worthless.
25. Cutting, sanitation—A cutting made to remove killed or injured trees for the purpose of preventing the spread of disease or insects.
26. Damping-off—The killing of young seedlings by fungi that cause decay of the stem or roots.
27. Decadence—Decline of vigor in forest stands.
28. Decay—The decomposition of wood substance by fungi.
29. Defect—Any irregularity or imperfection in a tree or wood products that reduces the volume of sound wood or lowers its durability, strength, or utility value.
30. Defect, hidden—A defect in a log or tree that cannot be observed before milling.
31. Defoliation—Reduction in the normal amount of foliage.
32. Deterioration—The physical decline or destruction of dead timber or forest products by wood-destroying fungi and/or phytophagus insects.
33. Dote—See Decay.
34. Durability—As applied to wood, its lasting qualities or permanence in service, with particular reference to decay.
35. Eradication—The elimination of a pest or the complete removal of a host for the controlling of a pest.
36. Fan, mycelial—A thin mycelial layer, composed of strands spread in a fan-shaped pattern, which occurs either over the surface of decaying timbers, or between bark layers, or between the bark and wood.
37. Felt, mycelial—A compressed layer, mat, or tough mass of fungous threads (mycelium).
38. Flag—A dying or recently dead leafy twig or branch which contrasts in color with the normal green foliage of the living tree.
39. Flux, slime—A persistent, fermenting exudate from trunk or branch wounds in trees.
40. Frost split—See Crack, frost.
41. Gummosis—(1) Partial or complete destruction of woody cells, the resulting mass being transformed into gum which occludes the newly-formed cavity and occasionally the cells adjacent to it. (2) A disease process in hardwood trees characterized by gum exudation.
42. Gum, wound—A dark amorphous substance produced in the wood of hardwoods and some conifers in response to injury, infection, or other irritation.
43. Hazard, decay—The risk of loss of wood volume from decay.

44. Heart, red—(1) A pronounced reddish coloration, often extensive, induced by fungi in the heartwood of conifers and a few hardwoods. (2) The common name of rot in balsam fir caused by *Stereum sanguinolentum*.
45. Heartwood, false—Wood abnormally colored through the action of fungi or bacteria or by the deposition of oxidation products; it resembles heartwood.
46. Heartwood, frost—False heartwood induced by unusually low temperatures.
47. Heartwood, pathological—Sapwood that has assumed heartwood characteristics prematurely as a result of wounding or irrigation.
48. Heartwood, traumatic—Pathological heartwood that has developed in the vicinity of wounds.
49. Heaving, frost—Lifting of seedlings or small plants as a result of expansion accompanying ice formation in frozen soil.
50. Hole, bore—A hole formed in a cell wall by the dissolving action of an advancing hypha of a wood-inhabiting fungus.
51. Injury, winter—See Winter drying.
52. Line, zone—A narrow colored line formed within wood in the process of decay by one or more wood-destroying fungi.
53. Mistletoe—A flowering plant of the family Loranthaceae, parasitic on trees and other woody plants.
54. Mold, snow—A molding or smothering disease of conifers developing under snow; contrast Blight, snow.
55. Mold, sooty—A dull blackish coating on leaves and other plant parts caused mainly by members of the Perisporiaceae (Meliolaceae).
56. Mycorrhiza—A symbiotic association between a fungus and the affected short roots of a higher plant.
57. Mycorrhiza, ectendotrophic—A form combining the features of ectotrophic and endotrophic mycorrhiza.
58. Mycorrhiza, ectotrophic—A form where the hyphae of the fungal component are entirely within the root cortex, and largely intercellular.
59. Mycorrhiza, endotrophic—A form where the hyphae of the fungal component are entirely within the root cortex, and largely intracellular.
60. Mycotrophic—(Of plants) having mycorrhiza.
61. Pad, mycelial—See Plaque, mycelial.
62. Parenchyma, wound—Parenchyma cells of irregular size, shape, and distribution resulting from injury.
63. Pathology, forest—Study of forest tree diseases and their control.
64. Peck—A decay localized in small areas, usually rounded or lens-shaped, surrounded by apparently sound or only slightly modified wood. *adj.*: Pecky.
65. Peck, bird—A small hole or patch of distorted grain from sapsucker (bird) attack on a living tree; it may be associated with discoloration, such as mineral streak.
66. Plaque, mycelial—A small, thin felt of mycelium formed between bark layers or between the bark and wood of trees.
67. Pocket, frost—Localized area where cold air settles.
68. Poplar, calico—Yellow poplar wood damaged by small holes made by insects and accompanied by long, black, greenish, or bluish streaks of stain.
69. Pseudomycorrhiza—A rootlet of a higher plant that shows a fungus in association but without evidence of pathogenicity and without typical mycorrhizal structure.
70. Punk—See Conk.
71. Punky—A soft, weak, often spongy wood condition caused by decay.
72. Red-belt—Winter drying confined to altitudinal zones; it approximately follows the contours. See Winter drying.
73. Resinosis—An abnormal exudation of resin or pitch from conifers.
74. Resistance, decay—See Durability.
75. Rhizomorph—A thread- or cord-like structure made up of hyphae.
76. Rib, frost—A pronounced linear protrusion of callus tissue formed by repeated attempts to heal a recurrent frost-crack.
77. Ring, drought—A zone of abnormal tissue, induced by water deficiency, within an annual ring in a stem.
78. Ring, frost—A brownish line or zone of abnormal tissue, induced by freezing, which extends partly or entirely around a stem, within an annual ring.
79. Ring, lightning—A band of abnormal wood in an annual ring, which results from mild lightning shock of the cambium.
80. Root, girdling—A root encircling part or all of the base of a tree and disturbing normal function.
81. Rot—Plant tissue in a state of decay.
82. Rotation, pathological—A cutting age determined by pathological considerations.
83. Sapwood, included—Concentric zones included in the heartwood, having the appearance and technical properties of sapwood.

84. Scorch—Injury to bark, flowers, foliage, or fruit by unbalanced nutrition, excessive sun, or hot, freezing, salt-laden, fume-laden, or unduly strong winds.
85. Scorch, bark—See Sunscald.
86. Shot hole—A leaf spot disease characterized by holes formed when dead parts drop out.
87. Spot, gum—An accumulation of gum-like substance occurring as a small patch; may occur in conjunction with a bird peck or other injury to the growing wood.
88. Spot, ink—Leaf disease of poplars caused by *Ciborinia* (*Sclerotinia*) spp.
89. Spot, leaf—Any one of several leaf diseases characterized by numerous isolated circular or angular lesions.
90. Spot, tar—Leaf spot caused by *Rhytisma* spp.
91. Spot, white—A whitish heat lesion on the stems of young tree seedlings, resulting from high soil temperatures at the ground line.
92. Stain, blue—A deep seated discoloration of wood resulting from fungus attack, predominantly bluish, but sometimes grayish, blackish, or brownish in appearance; confined almost exclusively to sapwood.
93. Stain, chemical brown—A brown discoloration of chemical origin that sometimes develops on wood in the course of air seasoning or kiln drying, probably from the oxidation of extractives in the wood.
94. Stain, fungus brown—A deep brown fungous discoloration of the sapwood, mostly in pines.
95. Stain, interior sap—Stain developing on the interior of sapwood boards and dimension stock during seasoning, without surface evidence of its presence.
96. Stain, iron-tannate—A bluish-black stain of oak and other tannin-bearing woods, caused by a chemical reaction between tannic acids and iron in cutting tools.
97. Stain, log—A stain present in the log before it is sawn into lumber.
98. Stain, mineral—An olive to greenish-black or brown discoloration in hardwoods, particularly hard maples; commonly associated with bird pecks and other injuries.
99. Stain, red—See Heart, red (1).
100. Stain, sap—Any stain that predominantly affects sapwood.
101. Stain, wound—A discoloration originating in, or developing as a result of, a wound in a tree.
102. Streak, mineral—Mineral stain occurring in streaks.
103. Streak, pitch—A well-defined accumulation of pitch (resin) in a more or less regular streak in the wood of certain conifers.
104. Streak, water—A dark streak in oak lumber caused by injury to the standing timber.
105. Strip, buffer—See Strip, protection.
106. Strip, protection—In blister rust control, a belt or zone outside the pine stand to be protected but included in the control unit and likewise freed of ribes.
107. Sunscald—Localized injury to bark and cambium resulting in wounds and caused by exposure of a stem to intense sunlight and high temperatures.
108. Sunscald, winter—Injury to bark and cambium resulting in wounds or cankers, localized on the side of the tree exposed to the midday and afternoon sun. Injury occurs as a result of freezing following the unseasonable warming of the bark by the sun in winter.
109. Tree, cull—Tree of merchantable size but unmerchantable because of defects or decay.
110. Volume, recoverable—The volume of a tree or stand, less cull volume.
111. Watercore—See Wetwood.
112. Wetwood—In a living tree, wood having an abnormally high water content and a translucent or water-soaked appearance.
113. Winter drying—The desiccation of foliage or twigs by dry winds at times when water conduction is restricted by low temperatures in the stem or root.
114. Zone, buffer—A controlled zone surrounding a protected area; it serves to minimize the disturbing effects of microclimate and other environmental factors exerted by adjacent areas.

TREE SURGERY TERMS

1. Cavity, bracing—Reinforcing side walls of deep wounds with rods.
2. Cavity, cleaning—Removing decayed and insect-bored wood in living trees.
3. Cavity filler—Material used to block the empty space remaining in a tree after removal of the deteriorated wood.
4. Cavity filling—Placing bulky and waterproof material in a deep wound.
5. Cavity, open—Deep wound within a cavity filler.
6. Cavity shaping—Giving a suitable form to deep wounds, in order to prevent water infiltration and to assure callus formation.
7. Cavity sterilizing—Application of an antiseptic coating on exposed sound wood in a cavity.

8. Cavity treatment—All operations of cleaning, shaping, sterilizing, dressing and filling deep wounds for the protection of wood in or to improve the appearance of living trees.
9. Chemotherapy—Direct treatment of tree diseases with chemical substances.
10. Impregnation, subsurface—Treating diseased trees by forcing a liquid therapeutant into the soil around the root system.
11. Surgery, tree—Maintaining health of trees by wound and cavity treatment and branch pruning.
12. Therapeutant—Chemical used for direct treatment of tree diseases.
13. Wound dressing—Substance applied to an exposed wood surface for the purpose of disinfecting or preventing entrance of wood-rotting fungi.

STUDIES OF THE DEVELOPMENT OF ZOSTERA MARINA L.

II. GERMINATION AND SEEDLING DEVELOPMENT[1]

A. R. A. TAYLOR[2]

Abstract

Seeds of *Zostera marina* L. are shed in Prince Edward Island waters during mid to late summer and germinate the following spring. Occasionally seeds germinate in nature before winter; they can be germinated during autumn and winter in the laboratory. Elongation of cells of the cotyledon and doubled-back axial hypocotyl pushes the embryo out of the seed coat. Cell increase plays no part in this or subsequent elongation of cotyledon and hypocotyl. Leaves emerge through the slit-like opening at the top of the cotyledonary sheath. Hairs develop from cells of the convex surface of the basal swollen hypocotyl; two adventitious roots grow from the cotyledonary node, develop root hairs, and anchor the seedling. Stelar tissues of the hypocotyl and cotyledon differentiate at germination. No characteristically thickened tracheary elements differentiate in hypocotyl or cotyledon. In the hypocotyl, phloem differentiates around the central xylem parenchyma and abuts externally on endodermis which develops Casparian strips. Xylem parenchyma differentiates adaxially against the phloem in the single cotyledonary strand. Differentiation of procambium and vascular tissues in the leaf primordia follows as these expand. Vascularization of the stem nodes is complicated by differentiation of strands to the roots and leaves. All internodes except the first have one pair of cortical bundles. The pair of lateral veins of the first true leaf differentiate in connection with the stele at that node; the lateral bundles from each later-formed leaf pass through one internode as cortical bundles and unite with the stele in the node below.

Introduction

This paper continues the presentation of the development of *Zostera marina* L. The specimens studied were from Prince Edward Island. It was established in the first paper in this series (7) that within the seed coat there is an embryo with a large, swollen, food-storing basal hypocotyl (Fig. 1). From the concave surface of this swollen part an axial structure extends toward the micropylar end. The lower part of this is the axial part of the hypocotyl. It continues as the plumule surrounded by the cotyledon. There is a sharp bend in this axial structure at the level of the plumule so that the tip of the cotyledon lies at the antipodal or basal end of the seed (Fig. 2). The plumule consists of a stem apex and three leaf primordia and is surrounded by the sheathing base of the cotyledon. Procambium is present extending as a strand from the micropylar end of the basal hypocotyl to and up the length of the axial hypocotyl; at the cotyledonary node procambium is differentiated in connection with the cotyledonary strand and that of the short epicotyl. The procambium in the epicotyl is differentiated into the first and second true leaf primordia. The cells of the tip of the cotyledon which had been meristematic in the developing embryo now appear to have degenerated.

[1]Manuscript received May 31, 1957.
Contribution from the Department of Biology, University of New Brunswick, Fredericton, N.B. This paper is from a thesis presented to the Graduate School of the Univeristy of Toronto, October, 1955, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.
[2]University of New Brunswick.

Time and Conditions for Germination

In the waters around Prince Edward Island fruits of *Zostera* mature from mid-July through August, but the majority of the apparently viable seeds are formed before mid-August. The ovary wall splits along its abaxial surface from the lower end to the stylar end to release the single seed. Most seeds are probably shed near the parent plant but sometimes branches, or whole flowering plants, bearing ripened fruit become detached, drift away, and shed their seeds at a distance.

Germination can occur immediately, but most seeds remain dormant on the sea bottom until after winter has passed. Tutin (8) in England, and Addy (1, 2) in the New England States, found that most seeds germinated in the spring; a few, however, germinated in the late summer or autumn of the season in which they were formed. Arasaki (3) in Japan found that germination occurred in nature 8 to 10 months after shedding (the greatest numbers germinating in February and March after being shed in the previous May or June). He was able to germinate seeds in the laboratory at any time. Many seedlings can be found in the late spring (May and early June) on Prince Edward Island shores, and since none were seen in late summer or early autumn (August to October) it has been concluded that delayed germination is normal here too.

Tutin (8) determined the percentage germination of small groups of *Zostera* seeds over the winter of 1935-36 and found that germination occurred both in light and in darkness at temperatures from 9–16° C. Arasaki (3) experimented with the conditions suitable for germination of *Z. marina*. He found that seeds of high specific gravity (sp. gr. = 1.19) had the highest rate of germination; seeds which had a soft, greenish hull could not be made to germinate. Germination seemed better in sea water of low salinity (chlorinity of 10 parts per mille or lower) although subsequent growth of the seedling was checked. The best germination rate was in water of low salinity at temperatures of 5–10° C. He concluded that salinity was more important than temperature, within the range 2–28° C. His study confirmed Tutin's observation that seeds will germinate in light or in darkness. He found "no effect" on germination when he experimented with pretreatments using a wide range of chemicals including acids, bases, salts, and organic substances.

Seeds from Prince Edward Island plants were held in open bottles in a refrigerator at 4° C. during the fall and winter months of the 1952–1953 season. From time to time samples were removed to room temperature and normal daylight; occasional seeds germinated within 2 or 3 days and became normal seedlings.

Morphological Changes in Germination and Development of the Seedling

Jensen (6) stated that the "double bent" part of the embryo grows out after the seed coat splits, that the sheathing leaf-like structure then rots away while roots grow out at its base and leaves push out at the top, and that

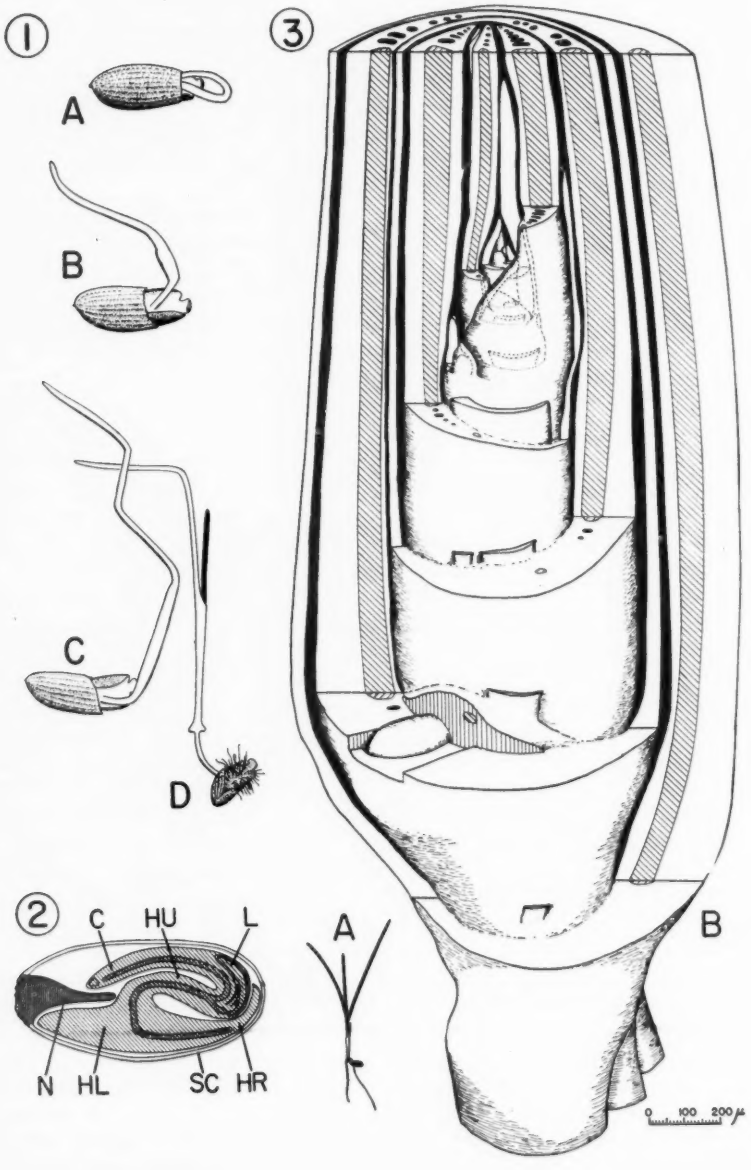
"root hairs" appear on the shield-like part of the embryo. Tutin (8) described the changes at germination as: a splitting of the testa near the plumule; an elongation of the plumule to produce the first leaf; the lateral extension of two small adventitious roots at the first node.

Arasaki (3) described the opening of a longitudinal slit in the seed coat at the micropylar end, through which the "tip of the coleoptile" appears and grows followed by the "endosperm"; then the "cotyledon" grows out, becomes green, and emerges from the fold as a small leaf. He described the "endosperm" as becoming smaller as the sprout grows.

All three workers briefly described the same processes although they had different conceptions of the nature of the parts of the embryo. The sequence of gross changes during germination of the seed of *Z. marina* is illustrated in Fig. 1. The split in the seed coat, which is the first indication of germination, usually starts at the micropylar end over the plumule and spreads toward the basal end. Variations have been observed in the position and extent of the split; in one seed it was found to be almost circumcissile.

Fig. 2 shows a near median longitudinal section of a mature seed prior to germination. The doubled-back axial part of the embryo elongates and pushes itself out of the seed coat (Fig. 1, A); this expansion is probably important in breaking the seed coat. The cotyledon elongates more rapidly than the hypocotyl and pushes the remainder of the embryo at least partly out of the seed. Further elongation, apparently greater on the inside of the curve, results in the emergence and straightening of the cotyledon (Fig. 1, B). Torsion of this axial structure is sometimes observed as it grows, particularly when the seedling is lying on a flat surface and over-balances because of its own weight (Fig. 1, C). A, B, and C in Fig. 1 show the same embryo at successively older stages. Fig. 1, D shows a considerably older seedling in which the first green leaf has extended through the opening at the top of the cotyledonary sheath. Hairs, resembling root hairs, have grown as extensions of the epidermal cells of the convex surface of the swollen, basal hypocotyl. The first pair of adventitious roots is evident (these were already organized at the cotyledonary node in the embryo within the seed, Taylor (7)).

The germination of specimens which had been stored for 4 months at 4° C. in sea water in the dark was observed in the laboratory. Emergence of hypocotyl, cotyledon, and plumule was complete in light and at room temperature 2 days after removal from the refrigerator in three out of four specimens (the fourth had its cotyledon still partly enclosed in the seed after 5 days). By the fourth day chlorophyll had appeared in the true leaves which were still enclosed by the cotyledon. Cotyledon and plumule continued to elongate, but by the seventh day the leaves had not yet emerged through the slit in the cotyledon. The measurements of one such specimen (Fig. 1, C) were: total length of the seedling to the tip of the cotyledon, 19.6 mm.; length of axial hypocotyl, 2.0 mm.; length of plumule (within the cotyledonary sheath), 5.9 mm.; length of cotyledon blade, 11.7 mm. (approximately). This specimen was then preserved for sectioning, but another similar seedling was 30 mm. long by the ninth day and had its first green leaf exposed 7 mm.


1
A
B
C
D
2
C
HU
L
N
HL
SC
HR
3
A
B
0 100 200 μ

Development now continues uniformly as the seedlings grow taller. New young green leaves, each ensheathed by the preceding, extend through the opening in the cotyledon. The youngest leaf exposed appears always as a short blade projecting from between the next most recent two. The leaves are in two ranks bending slightly alternately to right and left (Fig. 3, A). The first two roots elongate, grow down into the soil and develop root hairs. The second pair of roots is produced at the node of the first true leaf; they are not usually apparent until the third or fourth leaf has emerged.

The stem is vertical and very short at this stage; it consists of short internodes and is completely ensheathed by the cotyledon and young leaves. Where the seed has been buried by shifting sand or by silt deposition, the elongation of hypocotyl and plumule aid the penetration of the leaves to the surface of the soil. Occasionally in nature one finds seedlings with twisted and bent leaves, apparently injured in pushing up through the sand. The cotyledon becomes stretched thin by the expanding structures within; its blade early shows a deteriorated, 'withered' appearance and eventually the sheath is split from top to bottom. No cotyledon was ever observed with chloroplasts. Finally the whole cotyledon disintegrates.

The young seedling sketched in Fig. 3, A, was probably 3 weeks old, and certainly well established, when it was collected from the sand beach near the P.E.I. Biological Station on June 9, 1952. The large illustration (Fig. 3, B) is a reconstruction of the stem portion of this young seedling made by projecting and drawing serial transverse sections and then making this figure to scale from them. Two fully developed adventitious roots are present, one on either side of the cotyledonary node; two more are organized at the node above but not yet penetrating to the surface. The cotyledon is colorless, and has begun to degenerate at its tip, but the three young leaves which have grown out through the sheath are a bright green. There are five more young leaves or leaf primordia enclosed by the sheaths of the leaves which have already emerged. Branch primordia are present in the axils of the third, fourth, and fifth true leaves.

FIG. 1. A, B, and C: sketches of the same seed at 2-day intervals during germination in the laboratory, January, 1953 (× 3.3). D, sketch of a seedling with first leaf extended through the cotyledonary sheath, the first adventitious roots protruding at the cotyledonary node, and hairs developed from the convex surface of the swollen lower hypocotyl (× 2).

FIG. 2. Diagram of a median longitudinal section through a nearly mature seed; procambium is stippled (× 10). *C*, cotyledon; *HL*, lower part of hypocotyl; *HR*, 'radicle' end of hypocotyl; *HU*, upper part of hypocotyl; *L*, leaf; *N*, nucellus; *SC*, seed coat.

FIG. 3. A, sketch of the seedling sectioned to make the reconstruction in B (× 0.35). B, a reconstruction of part of this seedling, from the top of the hypocotyl to beyond the tip of the sixth leaf primordium (×55). Obscuring parts are shown cut away to reveal enclosed structures; broken lines show some structures obscured by overlying parts; diagonal hatching indicates procambium or vascular tissues; solid black shows air spaces, and spaces between the leaves. One root is shown as if exposed at the node of the first leaf (second node); only the bases of the intravaginal squamules are shown. The first branch axis is evident in the axil of the third true leaf.

Development of the Seedling Anatomy during and following Germination

Elongation of the Hypocotyl and Cotyledon

The first changes in the anatomy at germination are the elongation of cells of both cotyledon and hypocotyl, and the resumption of meristematic activity by the cells of the stem apex and its young leaf primordia.

The numbers and lengths of cells along the length of the hypocotyl in the mature embryo and the well developed seedling were compared in order to learn how the hypocotyl elongates. The cells could be counted and measured along the whole length of the hypocotyl of the embryo in the mature seed. In the seedling the elongated hypocotyl is twisted and curved, and is difficult to section in a truly longitudinal plane throughout its length. It was necessary, therefore, to count the numbers of cells in representative straight lengths, determine their average length, and estimate the number in the known total length of the hypocotyl.

In one representative, mature embryo, the total length of the hypocotyl was 1450 μ (measured along the convex surface of its curvature); the number of epidermal cells along this length was 90 (of 15 μ average length); the number of subepidermal cells, 57 (of 25 μ average length). In a well developed seedling, the total length of the hypocotyl was 21,000 μ; the average length of the epidermal cells was 220 μ, from which it was estimated that there were 95 epidermal cells in a longitudinal row; the average subepidermal cell length was 386 μ, and from this it was estimated that there would be 57 subepidermal cells in the length of the hypocotyl.

The whole axial hypocotyl increased in length by greater than 14 times. This was due to an increase in length of the individual cells already present in the embryo and not to an increase in cell number. At the same time the axial hypocotyl increased in width by one-third only; this was the result of a slight expansion in the transverse dimensions of the cells.

The basal swollen part of the hypocotyl does not increase in size; the only observable changes are the development of hairs from the epidermis on the convex surface and the gradual reduction of the abundant starch reserves. In the well developed seedling used in the above comparison, starch grains could be detected in occasional cells, usually near the margins of the hypocotyl.

Similarly, the cotyledon expands by an increase in the size of cells produced in the embryo rather than by the formation of new cells. This is illustrated in Table I, which gives dimensions and numbers of cells for the cotyledon in each of three representative specimens: A—an embryo in a mature seed; B—a newly germinated seedling with the first green leaf about to emerge from the cotyledonary sheath; C—seedling growing erect with two green leaves expanded.

No appreciable increase in number of cells was found along the length of the base and sheath of the cotyledon during development of the seedling, whereas cell length increased 18 to 39 times. The sharp bend in the base becomes straightened during the expansion of the seedling by a greater

TABLE I

EXPANSION OF THE COTYLEDON IN THE DEVELOPING SEEDLING

(A—embryo in mature seed; B—newly germinated seedling, first green leaf about to emerge from the cotyledonary sheath; C—seedling growing erect with two green leaves)

(1) *Sheath and base, longitudinal measurements*

	Sheath adaxial epidermis			Base adaxial epid.			Base abaxial epid.			Base abaxial subepid.		
	A	B	C	A	B	C	A	B	C	A	B	C
Length, μ	1220	5986	15,987	800	5930	15,870	400	5930	15,870	400	5930	15,870
Number of cells	208	170	121	80	90	88	61	60	62	31	30	42
Mean cell length	5.9	35	132	10	66	180	6.6	98	260	13	198	370
Mean cell thickness, μ	14	15	20–30	16	16	16–20	25	24	13	20	21	16–24

(2) *Sheath and base, transverse measurements*

	A	B	C
Thickness, μ	160	167	160
Number of cells thick*	2+5+6	2+5+6	3+5+6
Width, μ	340	610	1220

(3) *Blade, longitudinal measurements*

	Adaxial epidermis		
	A	B	C
Length, μ	1520	11,700	16,000
Number of cells	144	140	
Mean cell length, μ	10.7	90	
Mean cell thickness, μ	27	25	

(4) *Blade, transverse measurements*

	A		B	
	Midway	At bottom	Midway	At bottom
Thickness, μ	210	240	200	225
Number of cells thick*	3+5+4	4+6+5	4+5+5	3+5+6
Width, μ	260	260	308	310

*Note: 'Number of cells thick' gives the results in the form '$a + b + c$' referring to the number of cells across the mid-line through: *a*—adaxial part; *b*—vascular strand; *c*—abaxial part.

increase in the length of the cells on the abaxial side (originally the concave side of the curve) than on the adaxial side. The abaxial cells are stretched thinner at the same time. The base did not increase in thickness whereas it increased nearly four times in width and about 20 times in length.

In a similar but more detailed study of the growth and elongation of the coleoptile in the *Avena* seedling, Avery and Burkholder (5) also found that no increase in cell number occurred in the external epidermis; the tissue elongated by increase in length of individual cells. They noted, however, that the coleoptile elongated partly by an increase in cell numbers in its early stages, and that this increase was greatest in the subepidermis and increasingly less in the inner layers. The *Avena* coleoptile and *Zostera* cotyledon each function as a protective shield around the growing stem apex. The vascularization is different in each however. There are two matched coleoptilar vascular bundles in *Avena* and one cotyledonary vascular bundle in *Zostera*.

It is not possible to measure the blade completely in the upright seedling since it regularly becomes shrivelled and contorted soon after expansion of the first green leaves. A comparison of measurements from the newly germinated seedling with those for the embryo (Table I, 3) shows that the blade of the cotyledon expands more than seven times in length with no increase in number of cells along the length of the epidermis. There was no increase in thickness and but slight increase in width during the same period.

The expansion of the hypocotyl and cotyledon brings about the emergence of the germinating embryo from the seed. The continued growth and straightening of the cotyledon springs the tip of its blade free from the seed coat. The germinated embryo is then in a position to grow into a normal upright seedling. Epidermal hairs grow from the convex surface of the basal swollen part of the hypocotyl and anchor the seedling in the soil. The young green leaves push out through the slit at the top of the sheath of the cotyledon by growth and expansion; in Fig. 5, the first true leaf is shown about to push through the slit. The length of cotyledons is relatively uniform, but the axial hypocotyl varies in length especially with the depth to which the basal, swollen part is buried by shifting sand and silt. Seedlings found with the basal hypocotyl buried deeply have longer axial hypocotyls than those described and illustrated in this paper.

Activity of the Seedling Stem Apex

While the changes just described are occurring in the cotyledon and hypocotyl, the stem apex resumes the meristematic activity which was halted just before the embryo became dormant. The shape of the stem apex of dormant and of nearly mature embryos is determined largely by adjustments resulting from mutual compression amongst apex and leaf primordia within the confined space of the mature seed. This is not so in the young developing embryo, since the space within the ovule is not filled until late, nor is it true of the seedling once it has emerged from the confining seed coat (Fig. 4).

The form of the active seedling stem apex is similar to that of the active embryonic stem apex and both produce young leaf primordia in distichous sequence.

The size and form of the apex beyond the newest leaf primordium vary with the stage of the plastochron (the period from the initiation of one leaf primordium to the same stage of initiation of the leaf primordium at the next higher level). A detailed discussion of the form, organization, and activity of the stem apex will be deferred until the presentation of the development of the vegetative shoot, because the seedling stem apex does not differ appreciably from that of the mature vegetative shoot and, even more important, the activity and development of the stem apex can be better and more logically described over the total period of its life from epicotyl to mature growing vegetative stem.

Differentiation of the Anatomy of the Shoot and Root

The hypocotyl has a single provascular strand in the mature embryo. It was described (7) as differentiating very early in the formation of the swollen basal part and continuing to develop along with the development and growth of the axial portion at the base of the plumule.

A definite endodermis with well developed Casparian strips differentiates around the stele early in the growth of the hypocotyl; no Casparian strips were detected in the seed, but they are clearly visible in sections of hypocotyls from seedlings which have germinated but have not yet put forth their green leaves (i.e. such as in Fig. 1, C). There is no pericycle in the stele of the hypocotyl. Phloem differentiates from the cells between the endodermis and the xyloic procambial cells in the center of the strand. There are sieve tube elements, companion cells, and phloem parenchyma but no fibers. The number of phloic procambial cells increases during development of the hypocotyl by means of longitudinal anticlinal and, occasionally, periclinal divisions. Some sieve tubes lie next to the endodermis with their companion cells centrad or lateral to them.

In the center of the stele are two or three cells or, sometimes, a space resulting from their extension and disintegration. These cells are the only clearly xyloic cells of the stele, although they do not develop wall thickenings or other characteristics by which xylem cells are usually recognized. At the top of the hypocotyl, just beneath the cotyledonary node, these poorly differentiated xyloic cells are replaced by annularly, helically, or scalariformly thickened tracheary elements which have little or no lignin in their walls.

Formation of the cotyledonary node begins in the very young embryo before the elongation of the hypocotyl (7, Fig. 5), but very little procambium occurs at this level until late in the development of the embryo. Annularly, helically, or scalariformly thickened tracheary elements differentiate from the xyloic procambium during germination in association with advancing differentiation of the first stem internode and the first true leaf (Figs. 4, 6, and 8). Thickened tracheary elements first extend as a thin strand of three or four rows of cells from the top of the hypocotyl towards the first true leaf

primordium above (Figs. 4 and 6). Unthickened xyloic cells extend from the cotyledonary side of the node directly into the poorly developed cotyledonary vein; they are accompanied by phloem cells on the outside. The phloem differentiates directly from the hypocotyl into the cotyledon; additional original phloem of the node differentiates on the side of the xylem away from the cotyledon and continues into the midvein of the first leaf primordium (Fig. 4) through which it differentiates, on the abaxial side of the xylem, to the tip. The xylem also differentiates towards the tip of the young developing leaf. The remainder of the original phloem of the node differentiates on the sides of the xylem and slightly toward the cotyledonary face. Phloem then differentiates in the developing second node, internode, and leaf primordium.

The cotyledon has a single vascular strand or 'vein'. The procambial cells of this strand were formed in the embryo (7); they now enlarge in size and undergo differentiation into a definite phloic portion, consisting of parenchyma, sieve tubes, and companion cells, on the two flanks and abaxial face of the poorly differentiated xylem. These xyloic cells are interpreted as xylem parenchyma. It is made up of a layer of about six longitudinal rows of cells surrounding a single row of broad cells; these cells are homologous with the scalariformly thickened protoxylem tracheary elements which become stretched to extinction during the growth of the leaves. There is no clearly differentiated endodermis or pericycle; the single outer layer of cells, surrounding the others in the strand, might be considered endodermis, pericycle, or even phloic and xyloic parenchyma. There is no further development of these cells as the cotyledon matures.

The endodermis of the hypocotylar vascular strand extends into the lower part of the cotyledonary node and partly surrounds the vascular tissue. Early in the development of the seedling, cells in this nodal region, within the endodermis, undergo many divisions in various planes to form a compact mass; these cells have dense cytoplasm but are of abnormal procambial shape (Figs. 4 and 6). To either side of this region the young adventitious root apices begin to develop again and grow out of the axis. While this goes on, the new nodal cells differentiate into xylem and phloem (Fig. 10).

The main course of xylem is indicated by helically (generally) thickened tracheary cells. Xylem of the hypocotyl, and of the stele of each of the

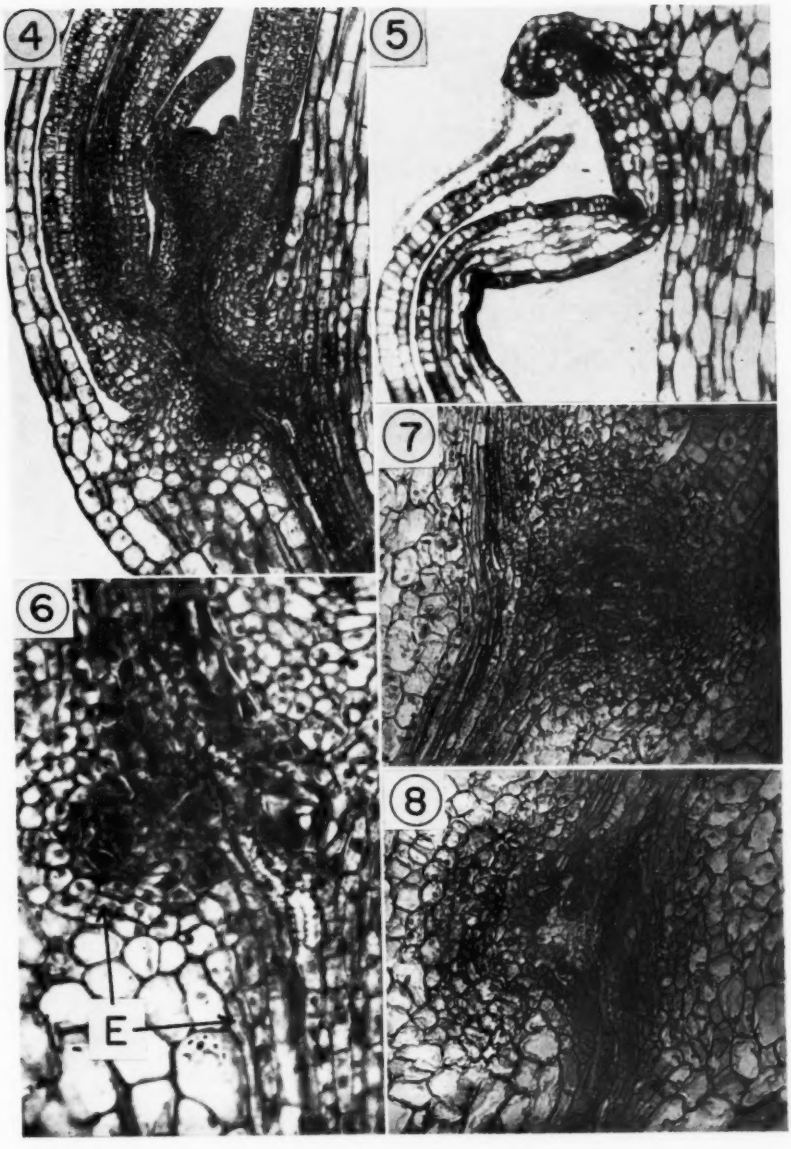
---

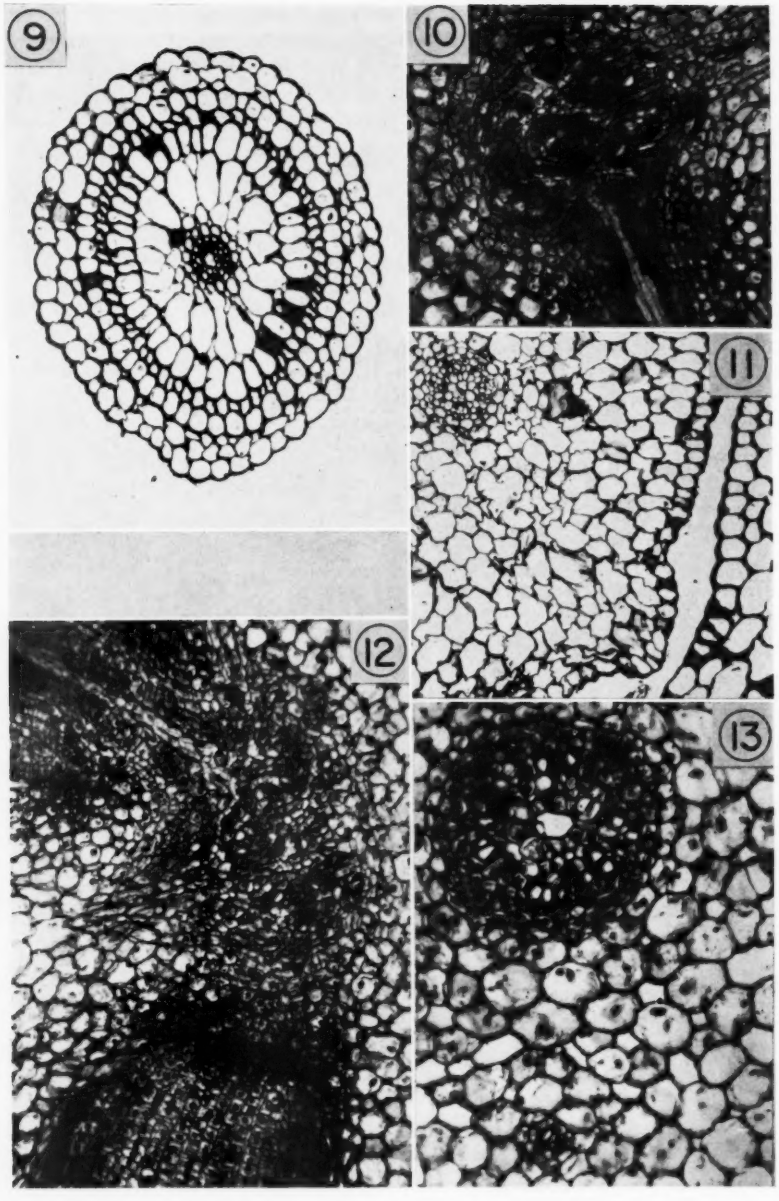
FIG. 4. Median longitudinal section through the apex of a newly germinated seedling (× 150). *This, and all succeeding photographs, are oriented so as to place the cotyledon towards the right.*

FIG. 5. Near-median longitudinal section through the top of the cotyledonary sheath of the seedling shown in Fig. 4. The tip of the first leaf is about to emerge through the slit (× 150).

FIG. 6. An enlargement of the cotyledonary node shown in Fig. 4. It shows the thickened-walled tracheary elements, the beginning of nodal plexus formation, and the endodermis with Casparian strips, E (× 400).

FIGS. 7 and 8. Near-median longitudinal sections through the fully developed nodes of the first leaf, above (Fig. 7), and the cotyledon, below (Fig. 8), of one young seedling (× 200).


4
5
6
7
8
E



Taylor—Can. J. Botany

two adventitious roots, becomes connected in the node. This additional xylem in the node becomes differentiated as characteristically thickened tracheary elements arching upward on each side and coming together by a gradual and somewhat irregular anastomosis to form the single strand of three or four rows of scalariformly thickened xylem elements in the center of the stele of the first stem internode. Phloem cells differentiate all around the xylem and between the two main xylem strands of the node; in places, other phloem cells differentiate intermingled with xylem cells in the anastomosing portion which is derived from the later developed nodal cells (Figs. 8 and 12).

The node of the first true leaf is no more than a position in the stem until after germination (this is in contrast to the cotyledonary node which shows procambium present in the embryo). There is no real appearance of internodes either, until after elongation occurs at germination. The internodes develop by elongation of cells of the stem beneath the leaf and in the adjacent tissues, and by the formation of new cells just above the node below. The cortical cell mass increases in part by occasional irregular cell divisions and in part by regular periclinal divisions in the primary thickening meristem in and beneath the abaxial side of the leaf base. Meristematic activity is small in extent and duration within the early-formed internodes; it becomes much greater in the later-formed parts of the stem.

The increase in length and thickness of the seedlings' internodes is given in Table II; it describes an embryo from a mature seed, a newly germinated seedling with its first green leaf ready to emerge from the cotyledonary sheath, and a well developed seedling growing erect with two green leaves extended and the seventh leaf primordium already initiated at the apex.

The young seedling stem grows most by increase in cell size and number in nodes and internodes back of the apex, not in the most recently formed parts. The cotyledonary node and the first internode quickly reach full size and maturity; they remain the smallest part of the stem. Succeeding nodes and internodes become increasingly larger; Table II does not give data on older seedlings in which this becomes apparent (mature stems may have internodes 1 or 2 cm. long).

The first internode differentiates with a definite stele in the center surrounded by cortical parenchyma (Fig. 11). The epidermis is unspecialized. The

---

FIGS. 9–13 show transverse sections cut from the young seedling illustrated in Fig. 3; compare with the diagrams in Fig. 14.

FIG. 9. A root from the cotyledonary node, cut near its base. The two outer layers of cells were derived from the root cap and act as a sheath (× 145).

FIG. 10. The cotyledonary node, to show the complex nodal vascular tissue; the cotyledonary trace is to the right, the vascular supply to one root is below (× 200).

FIG. 11. The first internode, to show the stele, and the cortex which has no cortical bundle (× 155).

FIG. 12. The node of the first leaf; the leaf trace extends part way to the left; the vascular connection with one root appears above (× 200).

FIG. 13. The second internode; note the stele, above, one cortical bundle, below, and the numerous small, schizogenous, cortical air spaces (× 285).

TABLE II

EXPANSION OF THE SEEDLING STEM

(A, B, and C as in Table I: A—embryo in mature seed; B—newly germinated seedling, first green leaf about to emerge from the cotyledonary sheath; C—seedling growing erect with two green leaves)

Internode	Stem	Length,* μ	Number of† cells long	Width,‡ μ	Number of‡ cells wide
1	A	41	6	135	14
	B	32	5	212	6+12+9
	C	640	10	412	8+13+9
2	A	48	6	65	10
	B	88	10	106	4+8+4
	C	263	16	320	9+12+10
3	A	40	6	55	7
	B	30	7	78	12
	C	160	15	215	9+12+10
4	B	37	6	62	7
	C	4	7	136	6+6+6
5	C	88	10	64	12
6	C	37	5	56	7
7	C	43	4	40	6

*Lengths were measured from the adaxial junction of one leaf with the stem to the similar position at the next higher level. These measurements actually consider the internode and node above together.

†Number of cells were counted up the length of the cortex of the internode and through the part of the node immediately above.

‡Widths were measured across the center line at mid-internode.

cortical parenchyma cells contain starch and amongst these cells small irregular air spaces occur; there is an endodermis with Casparian strips. The stele resembles that of the hypocotyl. Phloem parenchyma, sieve tube elements, and companion cells occupy the space between the endodermis and the xylem in the center. There are three tracheary elements, with the characteristic ring–scalariform wall thickening, occupying the very center; all tracheary elements observed appeared to be vessel elements although I cannot say unequivocally that there are no tracheids. This central core of tracheary elements is surrounded along its length by a sheath of large parenchyma cells, each almost as large in transverse dimensions as the whole group of tracheary cells. I interpret these cells as a sheath of xylem parenchyma. More xylem cells differentiate in the procambium as the upper end of the internode is approached until five to eight tracheary elements occur in the center.

The second node is associated with the first true leaf and becomes about 200 μ high. The vascular tissues differentiate here in a complex and intimate fashion (Figs. 7 and 12). The central xylem core differentiates through the node on a course which carries it into the young developing first leaf as its midvein (Fig. 4). Branches of xylem differentiate from later-formed procambial cells to the right and left to form large, looping anastomosing strands. These lie internal to the adventitious roots which develop just

outside the stele in the tissue at the base of the leaf (Fig. 12). Phloem is enclosed by these loops and more phloem differentiates around them. There are smaller loops of xylem, nearer the center of the node, that join the base of the large loops to the strand which passes to the first true leaf. A larger strand differentiates from cells toward the face of the node away from this first leaf. This strand continues as the xylem of the stele in the second internode. Phloem surrounds the xylem completely and phloem cells also accompany the xylem strand (on its abaxial side) that forms the midvein of the leaf (Fig. 4).

All the roots of *Zostera* are adventitious. It has been noted (7) that root apices are initiated at the cotyledonary node in the embryo. Earlier, this paper described how, following germination, these two roots grow out through the cortical tissues of the node and down into the soil where they develop root hairs. A single pair of roots develops at the node of the first leaf in the same manner. Each of these pairs of roots becomes closely associated with the complex anatomy of their respective nodes (Figs. 10 and 12). The small solid stele of the root differentiates in close association with that of the node. As the roots grow, a connection is made between the xylem of the looping anastomoses of the nodal complex and xylem of the root. Vessels with thickened walls occur near the stele of the stem; beyond this the xylem extends down the center of the stele of the root as three to five rows of thin-walled parenchyma. Phloem surrounds the xylem as a layer of sieve tubes, companion cells, and parenchyma, one to three cells thick, between it and the endodermis.

The young root grows out through the cortex of the stem destroying the tissues as it lengthens. It has a cortex of four or five layers of cells bounded by an epidermis which is, in turn, surrounded by a sheathing jacket produced by the root cap cells (Fig. 9). Development of the roots can be described in greater detail from the mature plant.

The course of the vascular tissues in the earliest formed parts of the seedling is shown diagrammatically in Fig. 14. Diagrams of transverse sections of the root (R), hypocotyl (A), and several levels of the shoot (B, C, D, I, II, and III) are also included in Fig. 14. Two small strands of procambium differentiate amongst the cortical cells on each side of the first leaf node. One strand is slightly below the other; the course of differentiation of the lower is inward from the lateral cortical bundle of the second internode, and connects with the stele in the first leaf node. The other procambial strand of each pair differentiates between the leaf and the stele very nearly paralleling the position of the young adventitious root. The direction of differentiation was not determined for this procambial strand in the seedling. These procambial strands become the lateral veins, one near each margin, of the first leaf; they differentiate acropetally in the leaf. Phloem differentiates in the cortical bundles of the stem and in the lateral veins of the first leaf, but xylem is confined to the stelar strand of the stem and to the median vein of the first leaf.

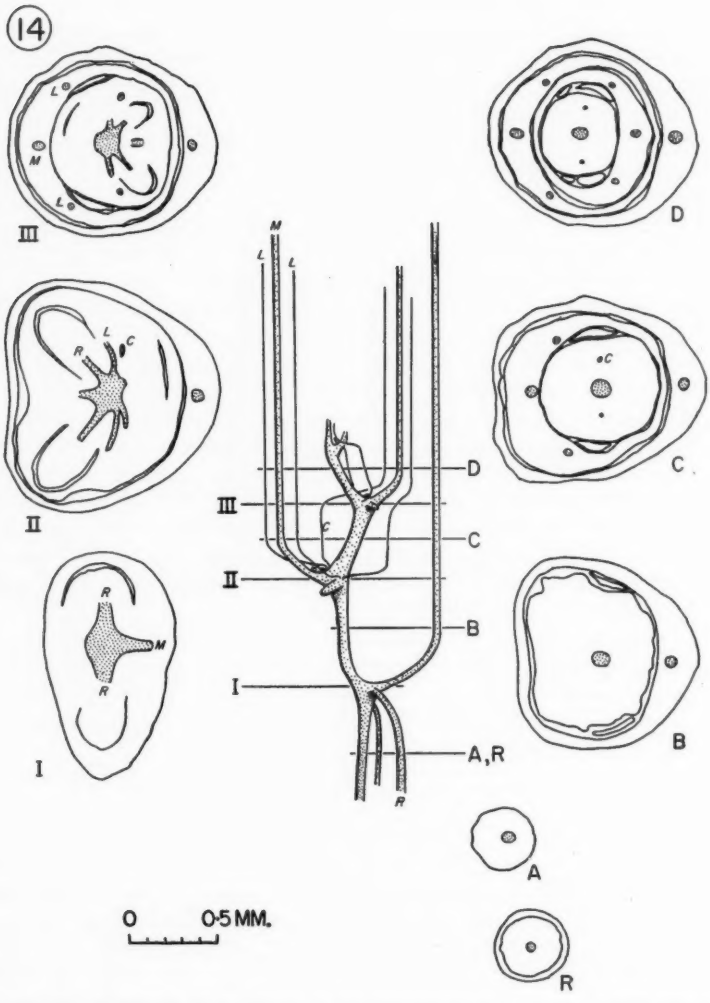


FIG. 14. A diagram of the course of the provascular and vascular system from the hypocotyl through the first three nodes and first three internodes of a young seedling, together with diagrams of transverse sections (× 30, approximately). These sections are of (from bottom to top): R, root from the cotyledonary node; A, axial hypocotyl; I, cotyledonary node; B, first internode; II, node of the first leaf; C, second internode; III, node of the second leaf; D, third internode. *C*, cortical vascular bundle; *L*, lateral vein or lateral leaf trace (cortical bundles of one node become lateral leaf traces at the next node); *M*, median vein or median leaf trace; *R*, root vascular supply.

The second internode differs from the first in having cortical bundles one on either side of the stele (Figs. 11, 13, and 14). It shares this characteristic in common with all succeeding internodes in the vegetative stem. The first internode is unique in possessing no cortical bundles. The cortical bundles of the seedling internodes are small and simple when compared with those of internodes of an old stem.

At the second leaf node procambium differentiates inward from the lateral veins of this second leaf, into the tissues at its base, and downward through the cortex of the second internode as the cortical bundles (Fig. 14). These are replaced in the third internode by the differentiation of another pair, associated with the third leaf; these join the stele at the second leaf node. Here, and at all later formed nodes in the seedling, no lateral bundles differentiate directly from the leaf to the stele of the same node as at the first true leaf node.

I do not propose to describe the development of leaves in detail in this paper, but it is worth noting that the development and final anatomy of the seedling leaves are essentially the same as in the leaves of the mature vegetative plant. The seedling leaves, however, are smaller in all dimensions and are simpler in structure. The first two have only three veins; later ones have the five veins typical of mature eastern Canadian plants (occasionally seven occur in older plants).

Intravaginal squamules, characteristic of so many aquatic monocotyledonous plants (Arber (4)), occur here also. They develop from cells on the sides of the stem in the angle between the leaf and stem (Fig. 3). Coherent files of cells form the scale. These structures are present within the sheath of the cotyledon and of all successively formed leaves.

Acknowledgments

Material was collected for this study while the author was employed as a seasonal investigator for the Fisheries Research Board of Canada at the Biological Sub-Station, Ellerslie, Prince Edward Island. I wish to express my thanks for the use of facilities there. I wish also to acknowledge with thanks the friendly criticism and advice of Professor H. B. Sifton of the Botany Department, University of Toronto, under whom this study was carried on.

References

1. ADDY, C. E. Germination of eelgrass seed. J. Wildlife Management, **11**, 279 (1947).
2. ADDY, C. E. Eelgrass planting guide. Maryland Conservationist, **24**, 16–17 (1947).
3. ARASAKI, M. Studies on the ecology of *Zostera marina* and *Zostera nana*. II. (*In Japanese.*) Bull. Jap. Soc. Sci. Fisheries, **16**, 70–76 (1950).
4. ARBER, A. On the "squamulae intravaginales" of the Helobiae. Ann. Botany (London), **37**, 31–41 (1923).
5. Avery, G. S., Jr., and Burkholder, F. R. Polarized growth and cell studies on the *Avena* coleoptile, phytohormone test object. Bull. Torrey Botan. Club, **63**, 1–15 (1936).
6. JENSEN, H. Zostera's spiring. Botan. Tidsskr. **17**, 162–167 (1890).
7. TAYLOR, A. R. A. Studies of the development of *Zostera marina* L. I. The embryo and seed. Can. J. Botany, **35**, 477–499 (1957).
8. TUTIN, T. G. The autecology of *Zostera marina* in relation to its wasting disease. New Phytologist, **37**, 50–71 (1938).

STUDIES ON THE INFLUENCE OF VARIOUS SUBSTANCES ON THE GERMINATION OF HELMINTHOSPORIUM SATIVUM SPORES IN SOIL[1]

S. H. F. Chinn and R. J. Ledingham[2]

Abstract

Fifteen groups, comprising 106 substances, were studied for their effect on the germination of conidia of *Helminthosporium sativum* Pamm., King and Bakke in soil. Spores usually germinated when substances from groups composed of natural products were included in the substrate. Of these substances, wheat germ, bran, certain oil meals, molasses, orange juice, and green plant tissues were very effective, whereas refined flours and commercial sugars were only slightly so. When wheat straw and roots of wheat plants were added to the soil the conidia did not germinate. In contrast to the above-mentioned groups, most of the substances in the acid, salt, vitamin, protein, amino acid, oil, saccharide, plant hormone, and miscellaneous groups were not effective. The exceptions in these groups were ascorbic acid, galactose, and raffinose.

Introduction

The behavior of fungal spores in soil has received relatively little attention because of difficulties in technique. Blair (1) has reviewed the techniques for direct examination of soil micro-organisms but none of these methods demonstrates adequately the behavior of specific fungal spores in soil. However, the buried-slide method, developed by the senior author (2), has proved satisfactory for germination studies with certain large-spored species of fungi. Spores of *H. sativum* were found to lie dormant in prairie soil but germinated when soybean oil meal was added. Furthermore, lysis of the hyphae occurred subsequent to germination. These findings, which were somewhat similar to those made by Mitchell *et al.* (6) with *Phymatotrichum omnivorum*, suggested to Chinn *et al.* (3) an approach toward the control of certain soil-borne plant diseases such as common root rot of wheat.

The desirability for further study, especially with regard to the eradication of spores of *H. sativum*, a cause of common root rot of wheat, has prompted a series of investigations. The present paper deals with a survey of the influence of various substances on the germination of spores of *H. sativum* in soil.

Materials and Methods

One hundred and six substances divided into 15 groups as shown in Table I were used in the experiment. The rates of amendment ranged from 0.015% for vitamin B_{12} to 10% for charcoal. Soil containing no supplement served as a check. The slide technique (2), with a slight change, was used to determine the effect of the various substances on the germination of spores of

[1]Manuscript received May 15, 1957.
Joint contribution from the Bacteriology Division (No. 439) and the Botany and Plant Pathology Division (No. 1608), Science Service, Canada Department of Agriculture, Ottawa.
[2]Bacteriologist and Plant Pathologist, respectively, Plant Pathology Laboratory, Science Service, Canada Department of Agriculture, University of Saskatchewan, Saskatoon, Sask.

H. sativum in soil. The change involved a step in the staining procedure; instead of being dipped in a solution of cotton blue and lactophenol the slides were placed on a level surface and the agar film sprayed with the solution. This procedure prevented slipping of the agar which often occurred when the slides were dipped. After staining, the slides were examined under the microscope. Percentage germination was calculated after examination of 50 conidia on each of two slides.

As indicated in Table I various substances were tested several times. Often, different samples of some of the substances were used and this may account for the discrepancies in some of the results shown in the table.

Results

The data in Table I show that spores of *H. sativum* did not germinate in the natural soil used in these tests. Germination occurred, however, following the addition to the soil of any of the 15 substances of the seed product group. The percentage germination ranged from 2% for pastry

TABLE I

EFFECT OF VARIOUS SUBSTANCES ON THE GERMINATION OF CONIDIA OF *Helminthosporium sativum* IN SOIL

Group	Substance	Rate of amendment %	Percentage germination			
			Trial 1	Trial 2	Trial 3	Average
Check	None	—	0	0	0	0
Seed products	Soybean oil meal	2	74	52	62	63
	Cornmeal	2	14	6	20	13
	Rapeseed oil meal	2	90	72	80	81
	Barley meal	2	56	25		41
	Linseed oil meal	2	28	20		24
	Rice meal	2	62	26		44
	Oatmeal	2	10	31		21
	Wheat (ground)	2	12	12	9	11
	Wheat germ	2	98	74	80	84
	Bran	2	76	54		65
	Whole wheat flour	2	70	39	35	48
	White flour (pastry)	2	0	4	2	2
	White flour (all purpose)	2	14	8		11
	Apple (fresh)	8	54			54
	Paprika	1	60			60
Stems and leaves	Wheat seedlings (green)	8	90	60		75
	Wheat plants (flowering stage)	8	40	40		40
	Wild mustard (green)	8	98			98
	Alfalfa (green)	8	100			100
	Sweet clover (green)	8	98			98
	Stinkweed (green)	8	90			90
	Crested wheat grass (green)	8	100			100
	Wheat straw (chopped)	2	0	0		0
	Alfalfa meal	2	70	100		85
	Sawdust	2	0			0
Tubers and roots	Wheat roots (seedling)	8	0	0		0
	Wheat roots (mature plant)	2 and 8	0	0	0	0
	Beets (table)	8	6	21		14
	Beets (sugar)	8	48	38		43
	Carrots	8	62	66		64
	Potatoes	8	70	8	33	37
	Alfalfa roots (tap)	8	69			69
	Sweet clover roots (tap)	8	76			76
Plant extracts and infusions	Carrot juice (canned)	4	68			68
	Orange juice (canned)	4	85			85
	Orange juice (fresh)	4	90	82		86
	Wheat seedling extract	8	96			96
	Wheat germ infusion	2*	82	90	89	87
	Soybean oil meal infusion	2*	71	78		75

TABLE I

EFFECT OF VARIOUS SUBSTANCES ON THE GERMINATION OF CONIDIA OF *Helminthosporium sativum* IN SOIL—*Concluded*

Group	Substance	Rate of amendment %	Percentage germination			
			Trial 1	Trial 2	Trial 3	Average
Animal by-products	Fishmeal	2	24			24
	Meatmeal	2	54			54
Excretory and secretory substances	Horse manure (fresh)	5	14	7		11
	Cow manure (fresh)	5	0	0		0
	Milk (cow)	10	20	0	4	8
	Urine	10	0			0
Acids and salts	Lactic acid	2	0			0
	Citric acid	2	0			0
	Tartaric acid	2	0			0
	Sodium citrate	2	0			0
	Sodium acetate	2	0			0
	Sodium pyruvate	2	0			0
Vitamins	Thiamine	0.5	0	0		0
	Biotin	0.025	0	0		0
	Riboflavin	0.5	0	0		0
	Nicotinic acid	0.5	0	0		0
	Pyridoxine hydrochloride	0.5	0	0		0
	Calcium pantothenate	0.5	0	0		0
	B_{12}	0.015	0	0		0
	Para-aminobenzoic acid	0.5	0	0		0
	Ascorbic acid	0.5	36	32		34
	Tocopherol	0.1	0	0		0
Vitaminized food	Aurofac (stock feed)	2	86			86
	Yeast whey (dried)	2	72			72
	Yeast extract	2	54			54
Proteins and amino acids	Vitamin free casein	2	0			0
	Casamino acid	2	0			0
	Albumin	1	0			0
	Proteose peptone	1	0			0
	Peptone	1	0			0
	Glutamic acid	0.5	0			0
	Aspartic acid	0.5	0			0
	Asparagine	0.5	0			0
	Glycine	0.5	0			0
	Lycine	0.5	0			0
	Alanine	0.5	0			0
Oils	Coconut	2	0			0
	Soybean	2	0			0
	Rapeseed	2	0			0
	Corn	2	0			0
Carbohydrates (crude)	Cane molasses	2	84	94	88	89
	Beet molasses	2	86			86
	Cane sugar (white)	2	9	1		5
	Beet sugar (white)	2	3			3
	Cane sugar (brown)	2	16			16
	Corn syrup	2	0	0		0
	Honey	2	0			0
	Starch	2	0			0
	Agar	2	0			0
Saccharides	Sucrose	2	0	0		0
	Arabinose	2	0			0
	Mannose	2	0			0
	Xylose	2	0			0
	Raffinose	1	16	0		8
	Lactose	2	0	0	0	0
	Galactose	2	12	12	22	15
	Melibiose	1	0			0
	Levulose	2	0			0
	Glucose	2	0	0	0	0
Plant growth hormones	Naphthalene acetic acid	0.1	0			0
	Indolebutyric acid	0.1	0			0
	Indoleacetic acid	0.1	0			0
Miscellaneous	Hypoxanthine	0.2	0	0		0
	Pyridine	0.5	0	0		0
	Krilium	0.5	0			0
	Charcoal	10	0	0		0
	Acetone	2	0	0		0

*Entire infusion from 2% of the dry substance.

flour to 84% for wheat germ. Whole wheat flour was intermediate in effect and all-purpose white flour quite low. Additions of rapeseed oil meal, bran, and soybean oil meal resulted in high percentages germination. Linseed oil meal, barley meal, rice meal, and oatmeal produced a moderate effect whereas corn meal and coarsely ground wheat had little influence on germination.

Green plants in the stem and leaf group all caused high percentage germination. Alfalfa meal had a similar effect. Chopped wheat straw and sawdust did not favor germination. Of the seven substances in the root and tuber group only wheat roots had no effect.

Plant extracts caused a high percentage of the spores to germinate. Infusions of wheat germ and soybean oil meal added at a rate equivalent to 2% of the dry substances also were highly effective.

Fishmeal and meatmeal had a moderate influence on germination whereas the excretory and secretory substances had little or no influence.

The six chemicals in the acid and salt group had no effect on germination nor did nine of the 10 vitamins tested. Ascorbic acid, however, applied at a rate of 0.5% resulted in 34% spore germination. The three substances in the vitaminized food group gave high percentages germination while the four substances in the oil group and 11 in the protein and amino acid group had no apparent effect.

Cane and beet molasses had a marked influence on germination whereas white cane and beet sugar had little or no effect. The less refined brown cane sugar caused 16% germination. Corn syrup, honey, starch, and agar were ineffective. Of the saccharides, raffinose and galactose had some effect. None of the plant hormones or the substances in the miscellaneous group favored germination.

Discussion

An interesting relationship was found among the various substances used in the experiment. In general, natural substances which were not refined or were only partially refined, favored germination. There were some exceptions, namely, straw, roots of wheat plants, and sawdust. On the other hand, most of the natural substances which were highly refined and most of the pure organic compounds were ineffective. Some exceptions also occurred here; commercial sugars, ascorbic acid, galactose, and raffinose caused some of the spores to germinate. None of the inorganic compounds promoted germination. At similar rates of amendment various parts of the wheat plant when added to the soil caused different percentages of the conidia to germinate, as illustrated by germination percentages of 84 for wheat germ, 65 for bran, 48 for whole wheat flour, 2 for pastry flour, and 0 for straw and roots. The stage of growth of the wheat plant also affected germination as shown by germination percentages of 75 for seedlings, 40 for plants in the flowering stage, and 0 for ripe straw.

Christensen (4) working with *H. sativum* noted improved spore germination in water and in soil extracts when plant tissue was added to the medium. Simmonds (7) and Chinn (2) studying the same fungus observed that the spores normally lie dormant in the soil and germinate only under certain conditions. Similar observations on spores of other fungal species were made by Dobbs and Hinson (5), who postulated the widespread existence in soil of a fungistatic factor. They noted also that certain nutrients placed in the soil cause spore germination. However, they realized that since many spores germinate freely in distilled water the nutrients were not necessarily responsible for germination. Data are not presented in this paper to support or refute the hypothesis of Dobbs and Hinson. Nevertheless, data from unpublished work of the authors indicate that a fungistatic substance present in the soil might well be responsible for the dormancy of *H. sativum*. The mode of action of the substances which induce germination is largely a matter for conjecture. It is suggested that the process is indirect and is effected through neutralization of the fungistatic factor, rather than by a direct effect on the spore.

Green manures have been long recognized as useful in controlling certain root diseases. Their role is incompletely understood, but has been related to changes in microbiological equilibria, changes in nutrients available to the host, influence on the pathogen, and other causes. The present study points to yet another mode of action of green manure and indeed of a number of organic substances. Such amendments result in germination of the spores, at least of *H. sativum*; in the absence of a host, the hyphae and mycelia perish. These considerations have potential value in the development of control measures for common root rot of wheat.

Acknowledgments

The authors wish to express their indebtedness to Dr. B. J. Sallans and Dr. H. Katznelson for valuable suggestions and constructive criticism of the manuscript and to Mr. E. J. Chatfield for technical assistance.

References

1. Blair, I. D. Techniques for soil fungus studies. New Zealand J. Sci. Technol. **26**, 258–271 (1945).
2. Chinn, S. H. F. A slide technique for the study of fungi and actinomycetes in soil with special reference to *Helminthosporium sativum*. Can. J. Botany, **31**, 718–724 (1953).
3. Chinn, S. H. F., Ledingham, R. J., Sallans, B. J., and Simmonds, P. M. A mechanism for the control of common rootrot of wheat. Phytopathology, **43**, 701 (1953).
4. Christensen, J. J. Physiologic specialization and parasitism of *Helminthosporium sativum*. Univ. Minn. Inst. Technol. Eng. Exp. Sta. Bull. 37 (1926).
5. Dobbs, C. G. and Hinson, W. H. A widespread fungistasis in soils. Nature, **172**, 197-199 (1953).
6. Mitchell, R. B., Hooton, D. R., and Clark, F. E. Soil bacteriological studies on the control of the phymatotrichum rootrot of cotton. J. Agr. Research, **63**, 535-547 (1941).
7. Simmonds, P. M. The influence of antibiosis in the pathogenicity of *Helminthosporium sativum*. Sci. Agr. **27**, 625-633 (1947).

TRANSLOCATION OF RADIOACTIVE SUGARS IN THE STEMS OF SOYBEAN SEEDLINGS[1]

C. D. Nelson and Paul R. Gorham

Abstract

Glucose-C^{14}, fructose-C^{14}, or sucrose-C^{14} were introduced into soybean seedlings through the cut petiole of one primary leaf. After 3 to 15 minutes the distribution of C^{14} throughout the plants was determined. Each of the three sugars was translocated as such without conversion to other compounds. Translocation was mainly downward from the point of introduction into the stem. Very little sugar was translocated to the primary leaf opposite the treated petiole or to the growing tip of the stem. Of the small amount of sugar that was translocated upward the bulk accumulated in the younger rather than the older trifoliate leaf.

When glucose-C^{14} or fructose-C^{14} were used the radioactivity in the stem decreased logarithmically from the point of introduction. The downward translocation of both sugars was impeded at the nodes. Translocation was not inhibited by 10^{-2} *M* potassium cyanide. These hexoses were translocated both upward and downward past killed sections of stems. When sucrose-C^{14} was used the radioactivity in the stem decreased linearly from the point of introduction. Translocation of sucrose was not impeded at the nodes and was inhibited by 10^{-2} *M* potassium cyanide. Sucrose was not translocated either upward or downward past killed sections of stems.

The minimum rate of translocation of both glucose and sucrose was the same. The rate was about 300 cm. per hour in the youngest seedlings and decreased with age to about 100 cm. per hour in the oldest seedlings.

Introduction

The study of translocation is hampered by the inability to introduce compounds directly into the conductive tissue of the intact plant. Foliar application as a method of introducing C^{14}-labelled sugars (4, 9) or growth regulators (2) has the inherent difficulty that translocation in the conductive tissue is obscured by the complications of penetration into and metabolic conversion by the surrounding leaf tissue. The method of studying the translocation of C^{14} from labelled photosynthate (1, 12) is no better since translocation is complicated by the rate of formation of adequate amounts of photosynthate and its movement to the conductive tissue. A simple method for introducing labelled compounds into the plant is to expose the vascular system by cutting off a leaf blade and to administer such compounds through the resulting petiole stump. This method is subject to the criticism that translocation may be different in a wounded and a normal plant but it was adopted for the reasons outlined above. In the present study, glucose-C^{14}, fructose-C^{14}, or sucrose-C^{14} were introduced into soybean seedlings and their distribution after translocation was determined. Also, the effects of cyanide, girdling, and age on translocation of these sugars were observed.

[1]Manuscript received May 31, 1957.
Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada.
Issued as N.R.C. No. 4433.

Materials and Methods

Seedlings of *Glycine max*, variety Flambeau, were grown in vermiculite in the greenhouse. Plants were used at one of three stages of development as follows: stage 0 plants, about 20 days old with stems from 25 to 30 cm. long and fully expanded primary leaves; stage I plants, 27 to 30 days old with stems 30 to 40 cm. long, primary leaves, and one fully expanded trifoliate leaf; and stage II plants, 30 to 35 days old with stems about 60 cm. long, primary leaves, and two fully expanded trifoliate leaves.

Sucrose-C^{14}, glucose-C^{14}, and fructose-C^{14} were isolated from 10 g. of tobacco leaves that had been allowed to carry on photosynthesis in 5 mc. of $C^{14}O_2$ for 18 hours (13). Specific activity of each sugar was 0.84 μc. per μM. carbon.

The seedlings were transferred from the greenhouse to the laboratory for translocation experiments. The blade of one primary leaf was excised in air with a razor. A small amount of liquid exuded and covered the freshly cut petiole stump. Some of this exudate may have been "sucked back" by any negative tension in the conductive vessels. Then the petiole stump was pushed into a piece of capillary tubing containing the solution of labelled sugar. This was taken as zero time for each experiment. The solution in the capillary tube was taken up at a rate of about 1 μl. per minute. After 3 to 10 minutes from 1 to 10 μg. of sugar had been taken up by each seedling. The petiole was removed from the solution and the plant was cut up into various pieces. Each piece was ground in liquid nitrogen in a mortar and the frozen, ground tissue was extracted with hot 80% ethanol. The ethanol-soluble extract was assayed for C^{14} and treated to paper chromatography and autoradiography as previously described (9).

In some experiments, tissues in a portion of the stem were killed by the local application of a fine jet of steam until the treated area became translucent. Plants treated in this way were left overnight in the laboratory before use in experiments.

Experimental and Results

The Form in Which Sugars Were Translocated

Soybeans that had been allowed to take up aqueous solutions of C^{14}-labelled sugars through the cut petiole of one primary leaf were divided into the various parts that are indicated in Table I. Chromatography and autoradiography of the 80% ethanol soluble extracts of each plant part showed that in times of 5 minutes or less, C^{14} was always recovered only in the compound that was introduced. In times from 6 to 9 minutes there was conversion of a small amount of sugar to other compounds in the leaves but not in the stems. Also, there was no radioactivity, even after 15 minutes in the 80% ethanol insoluble residues. Therefore, it is concluded that each sugar tested was translocated as such.

Distribution of Translocated Sugars Throughout Seedlings

Table I gives the distribution of C^{14}-labelled sugar in the various plant parts when either glucose-C^{14}, fructose-C^{14}, or sucrose-C^{14} was used as tracer.

TABLE I

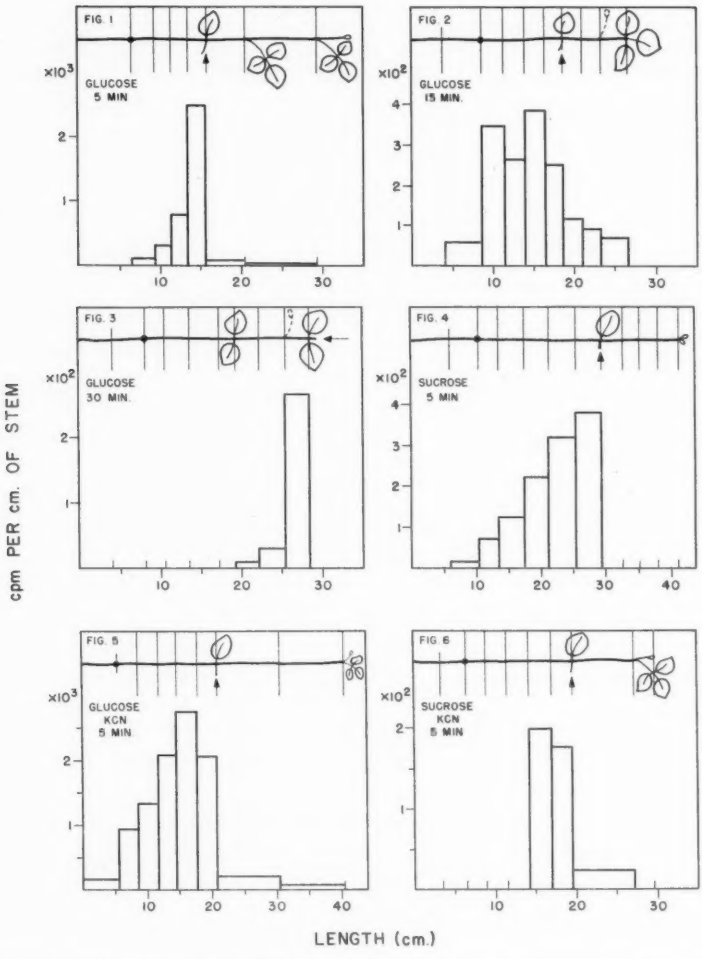
DISTRIBUTION OF C^{14} IN SOYBEAN SEEDLINGS AT DIFFERENT STAGES OF DEVELOPMENT AFTER UPTAKE OF GLUCOSE-C^{14}, FRUCTOSE-C^{14}, OR SUCROSE-C^{14} THROUGH THE CUT PETIOLE OF A PRIMARY LEAF

	% of total C^{14} recovered						
	Glucose			Fructose	Sucrose		
Plant part	Stage 0, 3 min.	Stage I, 9 min.	Stage II, 5 min.	Stage I, 6 min.	Stage 0, 5 min.	Stage I, 3 min.	Stage II, 3 min.
Stem tip	0	1.6	0	0.1	0	0	0
Upper trifoliate leaf and node	—	—	2.1	—	—	—	2.4
Stem between trifoliate nodes	—	—	0.5	—	—	—	2.2
Lower trifoliate leaf and node	—	2.1	0.6	4.2	—	0.2	0.1
Stem between lower trifoliate and primary nodes	—	7.2	0.3	1.0	—	0.3	2.3
Opposite primary leaf	0	0	0.8	0.6	23.1	11.1	0
Treated petiole and node	46.0	39.5	58.0	43.1	52.8	57.6	83.0
Stem between primary and cotyledon nodes	49.0	39.9	37.1	50.5	23.3	30.8	10.0
Stem between cotyledon node and root	5.0	9.7	0	0.5	0.8	0	0
Root	0	Trace	0	0	0	0	0
Total C^{14} recovered (mμc.)	182.0	24.5	73.5	45.4	121.0	11.5	81.0
Total C^{14}-sugar recovered (μg.)	6.5	0.9	2.6	1.6	4.1	0.4	1.9

About half of the labelled sugar in each plant was recovered from the treated petiole and node. A greater proportion of C^{14} was recovered from this part in the shorter times. In all plants the bulk of the labelled sugar was translocated downward from the primary leaf node. For example, in a plant given glucose-C^{14} for 3 minutes, 54% of the glucose was translocated downward while none was translocated upward (Table I). In longer times up to 11% of the glucose was translocated upward. In short times of 3 to 5 minutes there was no translocation to the stem tip of any of the sugars used. In longer times of from 6 to 9 minutes a small amount of sugar was translocated to this part.

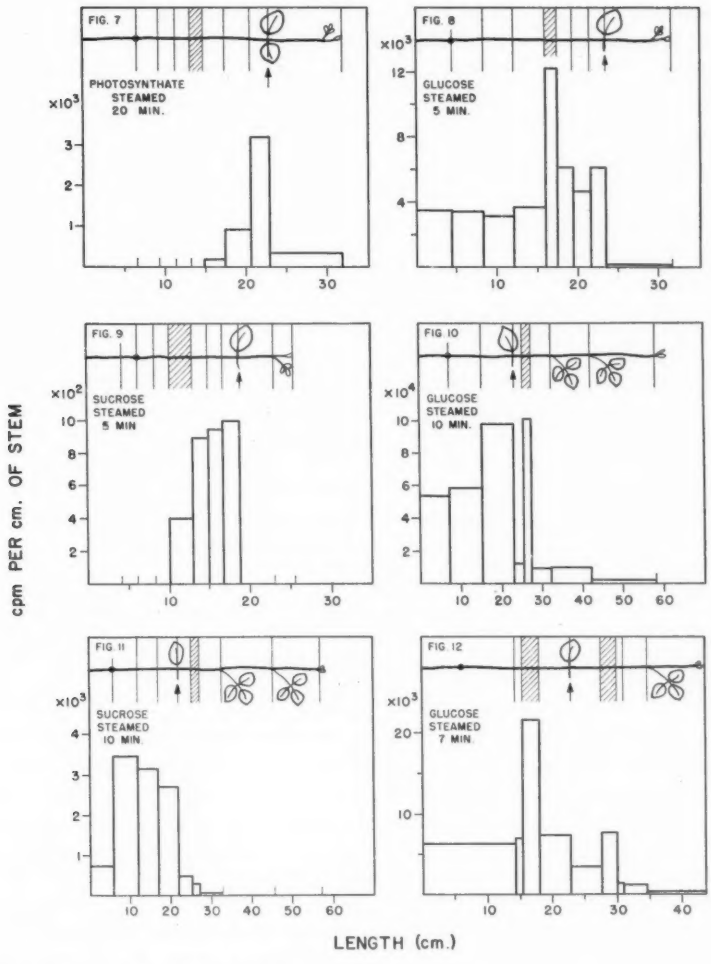
In all three stages of plant development the distribution of C^{14}-labelled sugar was the same for plants treated with either glucose-C^{14} or fructose-C^{14}. On the other hand, plants treated with sucrose-C^{14} had a slightly different distribution of C^{14} than those plants treated with the hexoses. There was considerable translocation of sucrose-C^{14} into the opposite primary leaf of stage 0 and stage I plants. With glucose- or fructose-treated plants of all stages and with sucrose-treated plants of stage II there was only a trace of C^{14} translocated to the opposite primary leaf.

In the plants that had two trifoliate leaves (stage II) there was always a greater proportion of C^{14} in the upper or younger leaf than in the lower or



FIGS. 1 to 12. Distribution of C^{14}-labelled sugar in soybean stems after uptake of sugar through the cut petiole. The arrow indicates the point of introduction of the sugar. Cross-hatching indicates the sections of stem that were killed by steaming. In FIGS. 2 and 3 the petiole of the trifoliate leaf is included rather than the stem-tip section. In all other figures only results for sections of stem are shown. Note that both of the scales, c.p.m. per cm. of stem and length (cm.), vary from one figure to another.

older leaf. Also the distribution of C^{14} among the leaflets of any one leaf was uneven. For example, in the upper trifoliate leaf of a plant treated with sucrose-C^{14} the radioactivity ratio for right leaflet : mid leaflet : left leaflet was 60 : 30 : 10. In different experiments and with different trifoliate leaves this ratio varied at random.



Translocation of C^{14}-labelled Sugars in Soybean Stems

Translocation of C^{14}-labelled sugars in stems was studied by cutting the stems into pieces 1 to 3 cm. long for C^{14} assay. Both total activity and distribution of radioactivity among compounds of the 80% ethanol soluble fraction were determined for each section of stem.

The results are shown in Figs. 1 to 12. Each figure shows a scale drawing of a soybean seedling with a histogram of radioactivity per cm. in each section of stem. Each figure represents typical results from the treatment of the following number of plants: Figs. 1 to 2 and 4 to 9, three to nine plants, one

or more in each stage of development; Fig. 3, three stage I plants; Figs. 10 to 12, four plants, two stage I and two stage II.

Translocation of Glucose-C^{14} and Fructose-C^{14}

The results of a typical short-term experiment when glucose-C^{14} was used as tracer are shown in Fig. 1. It is evident that the stem below the point of introduction of glucose was much more radioactive than the stem above this point. Also, the radioactivity decreased logarithmically from the point of introduction during downward translocation.

When glucose-C^{14} was administered through the cut petiole for 10 to 15 minutes, radioactivity per cm. of stem did not decrease logarithmically from the point of introduction (Fig. 2). Instead there was an accumulation of radioactivity in the internode below the primary leaf indicating that there was a partial barrier to translocation at the cotyledon node. There was no such barrier to upward translocation at the trifoliate leaf node. On the other hand, when glucose-C^{14} was introduced into the trifoliate leaf petiole there was very little translocation downward past the trifoliate leaf node (Fig. 3). It is concluded that the downward translocation of glucose was impeded at the nodes.

Similar observations were made when fructose-C^{14} was used as tracer.

Translocation of Sucrose-C^{14}

Like the hexoses, sucrose was translocated mainly downward (Fig. 4). However, in short times sucrose-C^{14} decreased linearly from the point of introduction and there was no indication of a barrier at the nodes. It is concluded that the translocation of sucrose was different from that of the hexoses.

The Effect of Potassium Cyanide on the Translocation of Sugars

When the cut petiole of a primary leaf was immersed in water or an aqueous sugar solution the rate of uptake was initially 1 μl. per minute. The rate of uptake decreased logarithmically over a period of 6 hours. If a small section was then cut from the exposed end of the petiole the initial rate of uptake was resumed. However, when 10^{-2} *M* KCN was introduced through the cut petiole the initial rate was 10 μl. per minute and there was no decrease in rate over a period of 6 hours. The changes in the petiole stump that were prevented by KCN were not investigated histologically.

The appearance of abnormalities in various parts of the plant gave an indication of the translocation of KCN. In a plant that was treated with 10^{-2} *M* KCN for 6 hours, necrotic areas appeared after 24 hours on the stem immediately below the point of introduction while the stem above this point appeared normal. This indicates that much of the KCN was translocated downward.

Fig. 5 shows the results of an experiment in which an aqueous solution of glucose-C^{14} containing KCN (10^{-2} *M*) was introduced in the stem. It is evident that KCN did not inhibit the translocation of glucose either downward or upward in the stem. However, two effects were always observed

when KCN was used in combination with glucose. First, radioactivity in the stem did not decrease logarithmically from the point of introduction. In several experiments there was no consistent relationship between total activity of stem sections and distance from the point of introduction. Second, there was no partial barrier to downward translocation at the cotyledon node. It is concluded that glucose moved downward in the dead tissues of the stem and that any control over this movement that existed at the cotyledon node was inhibited by KCN.

Similar observations were made for the translocation of fructose-C^{14} in 10^{-2} *M* KCN.

When KCN was introduced along with sucrose-C^{14} two effects were apparent (Fig. 6). The amount taken up was reduced by a factor of two and translocation of sucrose was inhibited. Therefore, sucrose was probably translocated downward in the living tissues of the stem.

The experiments with KCN confirm the conclusion that the translocation of sucrose was different from that of the hexoses when these sugars were introduced into the stem through the cut petiole.

Translocation of C^{14}-labelled Sugars past Killed Portions of Stems

It is well known that C^{14} from labelled photosynthate is not translocated past girdled or killed sections of stems (1). This has been interpreted to mean that organic compounds, especially sugars, are translocated in living tissues, probably the phloem. This was confirmed by the experiment shown in Fig. 7. Radioactivity, originating from 2 μc. of photosynthate in one primary leaf was not translocated downward past a steamed section of stem. It was also confirmed that C^{14} from photosynthate was not translocated upward past a steamed section of stem. Paper chromatography and autoradiography of the 80% ethanol soluble extracts of the stem sections revealed that the only detectable radioactive compound in these low activity experiments was sucrose.

Glucose-C^{14} (Fig. 8) or fructose-C^{14}, introduced into the stem through the cut petiole of a primary leaf, was translocated downward past a steamed portion of the stem. It is concluded that hexoses can be translocated in the dead tissue, probably the xylem elements. An experiment such as this does not show whether the hexoses can be translocated also in the living tissues. It is interesting to note that there was an accumulation of glucose-C^{14} or fructose-C^{14} in the killed section. When this section was examined it was found to be a dried out hollow cylinder of cells. It is possible that the labelled sugar leached out of the conductive tissue into the hollow cylinder of damaged cells.

Fig. 9 shows that sucrose-C^{14} was not translocated downward past a killed section of stem. It is concluded that sucrose was translocated only in living cells.

Since most of the sugar introduced through the cut petiole was translocated downward, experiments on upward translocation were carried on for times up to 10 minutes. Usually the C^{14} in the stems was recovered as the compound

administered but occasionally there was a slight conversion of the administered compound in experiments lasting longer than 5 minutes. Fig. 10 shows that the upward translocation of glucose-C^{14} past a killed section of stem was similar to the downward transport. The same results were obtained when a section of stem was killed both above and below the point of administration (Fig. 12). Similar results were obtained for the upward translocation of fructose-C^{14}.

A killed section of stem did not act as a total block to the upward translocation of radioactivity when sucrose-C^{14} was used as tracer (Fig. 11). The section immediately above the killed section contained a very small amount of C^{14}. The remainder of the stem above the killed area contained no radioactivity. This indicates that the upward translocation of sucrose was drastically impeded by the killed stem. The C^{14} in the stem section between the primary node and the killed section was recovered mainly as sucrose but with a trace of hexose. Unfortunately the amount of C^{14} in the section immediately above the killed section was too low to analyze by paper chromatography. It is possible that this small amount of radioactivity was translocated through the killed section as hexose which originated from the breakdown of the administered sucrose. It is felt that the general conclusion still holds, namely, that sucrose-C^{14} administered through the petiole or originating from labelled photosynthate was not translocated either upward or downward in dead tissues while glucose-C^{14} and fructose-C^{14} could be translocated in dead tissues.

Rate of Translocation of Sugars in Soybean Stems

A rate for the translocation of glucose can be determined from the data given in Fig. 1. In this experiment radioactivity was found in the stem 10 cm. below the point of introduction after translocation for 5 minutes. This gives a rate of 120 cm. per hour for the translocation of glucose in the stem of soybean. Since radioactivity must accumulate in any given stem section to a high enough level to permit detection, the calculation gives only a minimum rate.

Since radioactivity in the stem decreases logarithmically from the point of introduction, a second estimate of the rate of translocation of glucose can be obtained. Assuming that the front of radioactivity is at the locus in the stem where the logarithm of the radioactivity is zero, corresponding to one count per minute, the distance translocated in 5 minutes is 21 cm., which gives a rate of 252 cm. per hour. This method is not strictly valid since not enough is known to warrant the assumption that the front of radioactivity is at the locus in the stem where there is only one count per minute.

Table II shows that there was a relationship between the minimum rate of translocation and the stage of development of the plant. The minimum rate of translocation was high in the stage 0 plants, medium in the stage I plants, and low in the stage II plants. Also the rates of translocation for both sucrose and glucose were the same in plants at the same stage of development. Since sucrose cannot be translocated in dead tissues while glucose can, it might be expected that the translocation rates of these two compounds would

TABLE II

AVERAGE MINIMUM RATES OF TRANSLOCATION OF GLUCOSE-C^{14} OR SUCROSE-C^{14} IN SOYBEAN SEEDLINGS AT THREE STAGES OF DEVELOPMENT

	Glucose		Sucrose	
Stage of development	No. of determinations	Average minimum rate cm. per hr.	No. of determinations	Average minimum rate cm. per hr.
0	3	323 ± 32*	2	300 ± 7*
I	3	238 ± 40	3	220 ± 28
II	5	136 ± 24	4	130 ± 22

*Standard deviation.

be different. That the rates for the two compounds are the same may be a coincidence or may be an indication that glucose, like sucrose, is translocated in the living tissues of the unsteamed stem.

Discussion

It is of interest to compare the translocation of sugars introduced through the cut petiole of a leaf with the translocation of labelled photosynthate. However, too strict a comparison cannot be made for the following reasons: When C^{14}-labelled photosynthate is used as tracer, observation of translocation in the stem is obscured by movement through the leaf tissue into the conductive elements. Also, in photosynthate experiments, the amount of labelled material available for translocation increases throughout the experiment while the concentration of tracer administered through the cut petiole is kept constant throughout the experiment. With these differences in mind translocation in the two situations can be compared with respect to the distribution of tracer in various plant parts, movement in living tissues, and rate of translocation.

Distribution of Tracer

When labelled sugars were introduced through the cut petiole of the primary leaf, the distribution of C^{14} among the various parts of the plant (Table I) agreed with the observation made by several workers who used labelled photosynthate. Kursanov (cited in 6) using *Curcurbita pepo* and Nelson and Gorham (9) using *Glycine max* have shown that C^{14} from labelled photosynthate was translocated mainly downward, toward the roots. The latter workers also found that only a trace of sugar was translocated to the primary leaf opposite the leaf that fixed $C^{14}O_2$. Also, Rabideau and Burr (10) have found that no C^{13} could be detected in one primary leaf of *Phaseolus vulgaris* after the other primary leaf had fixed $C^{13}O_2$.

Movement in Living Tissues

Radioactivity from labelled photosynthate did not move past a killed section of stem. Chromatographic analysis of sections of stem showed that most of

the radioactivity was in sucrose. This can be interpreted to mean that sucrose was the main form in which carbon fixed in photosynthesis was translocated in the plant. The present experiments support this view in that sucrose, alone of the three sugars tested, did not move past a killed section of stem (Fig. 9), indicating that there is a mechanism in living cells selective for the translocation of sucrose.

Rate of Translocation

It is widely accepted that the rate of translocation of organic compounds in stems of plants is in the order of 100 cm. per hour (5, 12). The rate of translocation in the oldest soybean seedlings used in these experiments is in this order of magnitude. However, it must be pointed out that the rate of translocation may be much faster than this. For example, the rate of translocation was three times greater in the youngest than in the oldest seedlings used in these experiments. Also, rate measurements depend upon the accumulation of tracer in any section of stem in sufficient amount to permit detection. For this reason, only minimum rates can be calculated from this type of experiment. It is possible that a small amount of labelled material can move quite rapidly through stems and not be detected by ordinary means.

In experiments such as these where whole stem sections were analyzed there is the possibility that lateral movement obscured the observations made on downward translocation. For this reason it is difficult to correlate the linear pattern obtained with sucrose (Fig. 4) or the logarithmic pattern obtained with the hexoses (Fig. 1) and with photosynthate (11, 12) with translocation in the conductive elements. That the logarithmic pattern observed for the hexoses is due, at least in part, to lateral movement is indicated by the change in this pattern in the cyanide treated plants (Fig. 5).

It is difficult to explain the downward movement of glucose and fructose through a killed section of stem against the upward movement of water to the tops of the plant. The observation that the tops of soybeans remain turgid for several weeks after girdling proves that water does move upward from the roots under these conditions. Perhaps the hexoses do not move against the transpiration stream but in some conductive elements in the xylem while others are used for the translocation of water.

There is considerable evidence in the literature that translocation involves a metabolic component. This is contrary to the mass flow hypothesis proposed by Münch (8). Kursanov (7) has shown that the translocation of sucrose may be connected with respiration in the bark of *Caragana arborescens*. Hay and Thimann (3) indicate that there is a metabolic process controlling the translocation of 2,4-D. Willenbrink (14) has shown that the translocation of photosynthate through phloem of *Pelargonium* is affected by metabolic inhibitors. The present work is consistent with the view that the translocation of sucrose is a metabolic process. The mass flow hypothesis would predict that the rates of translocation of all compounds should be the same. Although this is indeed the case with the translocation of the three sugars, the rates are too high to be explained in this way.

No attempt has been made to explain the barrier to downward translocation of glucose that exists at the nodes of unsteamed stems. The removal of the barrier by KCN indicates that it is biochemical rather than anatomical in nature.

Conclusions

Glucose, fructose, or sucrose are translocated when introduced into the stems of soybean seedlings through a cut petiole. A selective mechanism which is sensitive to cyanide exists in the living cells for the translocation of sucrose. Glucose and fructose can be translocated in the dead tissue and translocation of these compounds is not inhibited by cyanide.

Acknowledgment

The excellent technical assistance of Gillian Kennedy is gratefully acknowledged.

References

1. CHEN, S. L. Simultaneous movement of P^{32} and C^{14} in opposite directions in phloem tissue. Am. J. Botany, **38**, 203–211 (1951).
2. DAY BOYSIE, E. The absorption and translocation of 2,4-dichlorophenoxyacetic acid by bean plants. Plant Physiol. **27**, 143–152 (1952).
3. HAY, J. R. and THIMANN, K. V. The fate of 2,4-dichlorophenoxyacetic acid in bean seedlings. II. Translocation. Plant Physiol. **31**, 446–451 (1956).
4. HULL, H. M. Carbohydrate translocation in tomato and sugar beet with particular reference to temperature effect. Am. J. Botany, **39**, 661–669 (1952).
5. KURSANOV, A. L. Circulation des matières organiques dans la plante et action du system radiculaire. *In* Essais de botanique. Vol. 1. 1954. pp. 142–153.
6. KURSANOV, A. L. Recent advances in plant physiology in the U.S.S.R. Ann. Rev. Plant Physiol. **7**, 401–436 (1956).
7. KURSANOV, A. L. and TURKINA, M. V. Dykhanie provodyashchikh tkanei i dvizhenie sakharozy. (Respiration of conductive tissues and the movement of sucrose.) Doklady Akad. Nauk S.S.S.R. **85**, 649–652 (1952).
8. MÜNCH, E. Versuche über den Saftkreislauf. Ber. deut. botan. Ges. **45**, 340–356 (1927).
9. NELSON, C. D. and GORHAM, P. R. Uptake and translocation of C^{14}-labelled sugars applied to primary leaves of soybean seedlings. Can. J. Botany, **35**, 339–347 (1957).
10. RABIDEAU, G. S. and BURR, G. O. The use of the C^{13} isotope as a tracer for transport studies in plants. Am. J. Botany, **32**, 349–356 (1945).
11. SISLER, E. C., DUGGER, W. M., and GAUCH, H. G. The role of boron in the translocation of organic compounds in plants. Plant Physiol. **31**, 11–17 (1956).
12. VERNON, L. P. and ARONOFF, S. Metabolism of soybean leaves. IV. Translocation from soybean leaves. Arch. Biochem. Biophys. **36**, 383–398 (1952).
13. VITTORIO, P. V. The use of carbon-14 in the study of starch and sugar synthesis in detached tobacco leaves. Ph.D. Thesis, Queen's University, Kingston, Ontario. 1953.
14. WILLENBRINK, J. Uber die Hemmung des Stofftransports in den Siebröhren durch lokale Inactivierung verschiender Atmungsenzyme. Planta, **48**, 269–342 (1957).

CYTOTAXONOMY OF CAREX SECTION CAPILLARES[1]

ÅSKELL LÖVE,[2] DORIS LÖVE,[2] AND MARCEL RAYMOND[3]

Abstract

The small section *Capillares* of the genus *Carex* has been studied from the cytological and taxonomical points of view. It comprises nine distinct species, one of which (*C. Boecheriana*) is raised to specific status in this paper. Three species include two distinct subspecies each; two varieties and three formae are accepted for two species respectively. The chromosome numbers of the taxa involved are $2n = 18$, 36, 38, 40, 54, 56, and 58. It is pointed out that the chromosomes of *Carex* have a polycentric or diffuse kinetochore and that this explains the aneuploid variations in number known to be typical of the genus. Karyotypic analysis of the chromosome complements of the section *Capillares* revealed that in the basic diploid set of $2n = 18$ chromosomes four units are long, 10 are medium and about half the length of the long ones, while four are small and about half the length of the medium ones. This frequency of size classes is also met with in the plants with $2n = 36$ and 54 chromosomes. Those taxa with $2n = 38$, 40, 56, and 58 chromosomes show, however, such a deviation in the frequency of the size classes that it seems logical that these numbers have been produced by a secondary fragmentation of the chromosomes. On the basis of karyotypic analysis of this section and other groups of the genus it is concluded that the primary basic number for the genus *Carex* in particular and the family Cyperaceae in general must have been $x = 5$, all other numbers having been derived mainly by fragmentation of chromosomes with a polycentric or diffuse kinetochore. This phenomenon of partial agmatoploidy is widespread in Cyperaceae, whereas complete agmatoploidy is typical of the closely related Juncaceae.

I. Introduction

Critical species and races are known from within all families of vascular plants on all continents, but there are only few genera with a higher number of disputed or otherwise confused taxa in different parts of the world than the genus *Carex*. This large, natural, and amphimictic genus is represented by over 2000 more or less distinct species from all regions of both hemispheres, although it prefers alpine and subarctic conditions even up to 5–6000 meters height in the Himalayas; *C. atrofusca* Schkuhr has been collected at 5127 m.s.m. in Tibet (279) and *C. Moorcroftii* Falc. and *C. nivalis* Boott at 5475 m.s.m. in the Karakorum (282). It covers larger areas in the Arctic than any other plant group. The genus seems to favor moist conditions, although not wholly abandoning dry locales, and there are strong indications that its species originally preferred dry soils. There are maritime species of the genus growing in constantly or periodically salt water, others prefer deep lakes or boggy mires far away from the ocean, while still others thrive only on dry rocks of high and arctic mountains. Some taxa show no climatic preferences and are ecologically indifferent. A few species of *Carex* are anthropochorous, and although some species find the very rich soils of birdcliffs most enjoyable, most taxa avoid the soils characterizing the neighborhood of dwellings. Some

[1]Manuscript received April 8, 1957.
Joint contribution from l'Institut Botanique de l'Université de Montréal and the Montreal Botanical Garden.
[2]Institut Botanique de l'Université de Montréal, 4101 est, rue Sherbrooke, Montréal 36, Qué.
[3]Montreal Botanical Garden, 4101 Sherbrooke Street East, Montreal 36, Que.

species are tufted, others single-stemmed or creeping, and their height may vary from less than a centimeter to over two meters. Most of the northern species are narrow-leaved or grasslike, whereas some of the supposedly primitive subtropical and tropical taxa are characterized by broad and very large leaves. Most of the species are monoecious but some are dioecious, and the generic variations in the size and form of the peculiar fruit are almost incomprehensible even though its variability within each taxon is remarkably narrow. By aid of morphological differences the genus has been subdivided into a number of more or less natural subgenera and sections, but attempts to separate some of these taxa as genera do not seem to have been very successful.

In addition to possessing all the pecularities just mentioned, the genus *Carex* is known to be exceptional also in its cytology and embryology. The first students of the embryology and cytology of the genus observed that not only do three of the four macrospores disintegrate as in other higher plants but also three of the four microspores are always aborted so that only one pollen grain comes out of each tetrad. About 300 species have been studied from most sections and all the subgenera since the first observations by Juel (159), Stout (340), and Heilborn (126) were reported; exact countings of the chromosome number, mainly based on meiotic divisions, have been published by Stout (340), Heilborn (127–135), Tanaka (347–373), Wulff (407–410), Böcher (27), Okuno (275, 276), Wahl (395), Levan and Löve (196), Levan (in 214), Flovik (91), Löve and Löve (216, 217, 220), Ehrenberg (85), Harling (111), Tarnavschi (375), Sörensen and Westergaard (in 217), P. H. Clausen (in 379), Pólya (289), Knaben (163), Holmen (141), Reese (304), Davies (73, 74, 75), Löve (211, 212), and Skottsberg (329). These studies all have emphasized that although chromosome numbers from $2n = 12$ to $2n = 112$ are characteristic of different species of the genus, and related species often reveal small differences in chromosome number, these numbers are not attributable to any definite and common basic number other than $x = 2$. Polyploidy is so rare in the group that it was long thought to be entirely absent. And although even small variations in the number and type of chromosomes is found to be of a certain significance at the specific level, these variations lend little support to subgeneric and sectional subdivision, nor do they take exception to it.

It was pointed out by Heilborn (128) and supported by all later authors, that the size of the chromosomes of *Carex* usually diminishes with an increase in number, or, perhaps more correctly, the frequency of large chromosomes decreases with an increase in chromosome number. This very exceptional phenomenon was attributed to fragmentation or transverse division of single chromosomes of the set, thus allowing a dysploid addition in chromosome number. Until recently such a fragmentation was not easily conceivable since the concept of single and constant centromere did not allow for the survival of acentric fragments. Now it is known, however, that such a fragmentation is possible in organisms with polycentric or diffuse kinetochores. This is a rare but evolutionarily very significant phenomenon still unknown

from higher plants other than a few families of Monocotyledons. It allows for additions in ploidy other than polyploidy and has been most appropriately termed agmatoploidy (233) and, less befittingly, endonuclear polyploidy (259) and pseudopolyploidy or even agmatopseudopolyploidy (17).

Still another cytological process has been reported to distinguish *Carex* from most other higher plants. Wahl (395) presented preliminary evidences indicating the occurrence of postreductional meiosis in *Carex*, a phenomenon later observed also in *Luzula* and suggested to be connected with the occurrence of diffuse or polycentric kinetochores (49, 51, 52, 229, 232, 233). This important report by Wahl (395) has been doubted by some authors (cf. 18) but confirmed by others (75), while other workers studying Cyperaceae have either been non-committal (110) or noted only regular behavior without any evidence of equational divisions during the first phase of the meiotic divisions (325, 347–373).

The subgeneric evolution of *Carex* cannot be grasped at present from the available cytological material, nor is it possible to find any satisfactory explanation for the evolution of the different sections on the basis of already published data. This is perhaps caused by lack of systematic collection of comprehensive material from any one section rather than by a lack of any general tendency, but it is also likely that the different sections—and even the different taxa of the same section—may have evolved with the aid of different mechanisms, making insufficient any one explanation for the group as a whole. Only a detailed study of all the representative taxa of all sections will give satisfactory information as to the variations in micro- and macro-evolutionary mechanisms responsible for the multitude of this primitive genus.

The present paper is intended to be a small contribution to the studies of the evolution of the genus *Carex* in particular and of the family Cyperaceae in general. It is a report of combined cytological and taxonomical studies of one of the smallest sections of the *Carex* subgenus, the *Capillares* section, which is a group confined to the northern hemisphere and includes variable species with wide distribution as well as taxa with limited area and small variability. The section is circumpolar boreal–arctic because of one of its species only, but is otherwise most variable in the Pacific area of northern Asia and subarctic–arctic North America. It certainly is an old and well-established section without clear connections to other groups of the genus, and although many of its species are old and conservative, others are apparently recent and still in course of evolutionary change.

II. The Taxa of Section *Capillares*

As in other critical groups, several taxa at different levels have been described within what now is regarded as the distinct and natural *Capillares* section, and the group as a whole has been placed in various major taxa in the past. Linnaeus (204) placed it with a few other species in a group described as "spicis sexu distinctis: femineis pedunculatis". Koch (164, 165) grouped it in the *Legitimae*, Fries (96) classed it in the *Heterostachyae–Tristigmaticae*,

whereas Cosson and Germain (69) included it in the subgenus *Eucarex*. This latter classification was accepted by Kükenthal (181) and other modern monographers of the genus, but according to the International Code (193) the correct name of the subgenus is *Carex*, since it includes the type species of the genus, *C. hirta* L. (cf. 40). Ehrhart (86) placed the Linnaean species *C. capillaris* in the group or genus *Caricella*, Drejer (79) listed it in the section *Hymenochlaenae*, and Fries (96) included it in the section *Strigosae* (cf. also 6). Meinshausen (241) grouped the Linnaean species and other related and later-described taxa together with a number of morphologically somewhat similar but probably unrelated taxa in the section *Chlorostachyae*. Tuckerman (385) included it in the section *Sylvaticae* Boott, although Boott (37) declared that its "affinity is with the *Strigosae*", and then Bailey (12) referred it to the somewhat more restricted section *Flexiles* Tuckerman (cf. also 139). The real distinction of the group was not understood until Ascherson and Graebner (8) classed the only species known from Central Europe in the section *Capillares* and pointed out especially that a grouping of it together with *C. silvatica* and *C. strigosa* appeared to be purely artificial. This view is strongly supported by morphological and, particularly, cytological data.

Although Kükenthal (181) accepted the view of Ascherson and Graebner (8) as to the classification of *C. capillaris* and its relatives in a distinct group, he regarded it only as a subsection of *Hymenochlaenae*. Most later authors, however, have accepted the sectional status of *Capillares* (cf. 4, 224, 270, 300), but even though Kreczetovicz (177) concurs in this view, he creates the sectional name *Caricella* (Ehrh.) Krecz. for the group and includes in it nine species from within the Soviet Union. He also goes one step further, since he separates within the section the three series ("riad") *Nematodae*, *Chloroticae*, and *Chalcandrae*. This latter subdivision is, however, artificial rather than natural, and it is certainly not needed for such a comparatively small group of species.

It would lead too far here to list and discuss all the many useless names of taxa of this group proposed by different authors in the past; such names of interest for the synonymy of the different taxa recognized by the present writers will be listed on a later page. But in order to make it possible to name the ones used for the cytological investigations a mention will be made here of the taxa recognized by a few recent authorities making serious efforts to classify the section into its natural entities. Although some of these names will be used in this paper, their classification into categories is not necessarily the one accepted by the present writers, as will be evident from the taxonomical review at the end of the paper.

In his magnificent monograph of the genus *Carex*, Kükenthal (181) recognized 11 taxa of the *Capillares* group and placed them as the four species *C. Sedakowii* C. A. Meyer, *C. capillaris* L., *C. koreana* Komarov, and *C. Williamsii* Britton. The only taxon of which intraspecific variations are listed is *C. capillaris*, which according to Kükenthal (181) includes the typical variety α *tenuior* Drejer as well as the varieties β *nana* (Cham.) Kük., γ *Ledebouriana* (C. A. Meyer), and δ *Regeliana* Kük. For the typical variety

Kükenthal (181) lists as synonyms *C. pendula* Genersich non Huds., *C. plena* Clairv., and *C. chlorostachys* Stev., but divides it into three formae: f. *minima* Beck from the Alps, which is said to be synonymous with f. *alpestris* Norman from Scandinavia; f. *major* Drejer, which is the plant depicted in Flora Danica t. 2374, Fig. 3, and regarded as synonymous with *C. capillaris* ssp. *Karoi* Freyn and var. *elongata* Olney; and f. *saskatschewana* (Böckeler), wrongly attributing its transfer from the specific level to that of a forma to Bailey (13). The var. *nana* from Alaska is said to be synonymous with *C. nana* Cham. and *C. Chamissonis* Meinsh., but includes also the forma *Krausei* (Böckeler) Kük. which is *C. Krausei* Böckeler. The var. *Ledebouriana* is said to antedate var. *Ledebourii* F. Schmidt and is also identified with the species *C. lenaeensis* Kük. described by Kükenthal (180) himself a few years earlier. And the var. *Regeliana* is said to be a plant from Turkestan synonymous with *C. ferruginea* Meinsh.

The treatment by Kreczetovicz (177), including only Russian material, differs from that by Kükenthal (181) in the concept of species as well as in some basic interpretations. The species *C. Sedakowii* is retained, and so are also *C. koreana* and *C. capillaris*, the latter of which is restricted to the arctic var. *tenuior*. But the f. *major* here is made synonymous with both the species *C. chlorostachys* Stev. and *C. Karoi* (Freyn) Freyn; the var. *Ledebouriana* is regarded as identical partly with *C. lenaeensis* Kük. and partly with *C. Ledebouriana* C. A. Meyer both of which are retained as equivalent species; and the var. *nana* is listed as the species *C. Chamissonis* Meinsh., since *C. nana* Cham. has an older homonym. An additional Asiatic species is *C. Novograblenovii* Komarov (171) from northeasternmost Asia. But the var. *Regeliana* of Kükenthal (181), which had been shown to be a species, *C. Regeliana* (Kük.) Kük., closely related to *C. misandra* R. Br., by Kükenthal (in 207), is excluded from the section and included in the section *Orphinascus* Boern. Naturally, Kreczetovicz (177) does not mention the North American taxa when they are not also known from within the Soviet Union.

In his Flora of Alaska and Yukon, Hultén (149) evaluates the Russian species and compares them with his Alaskan material. He finds that while *C. Sedakowii*, *C. Karoi*, and *C. koreana* are very distinct Asiatic species, some of the other Russian taxa are less clear-cut. He points out that the Alaskan *C. capillaris* usually is much taller than in Scandinavia and at least similar to *C. lenaeensis* (sensu Kreczetovicz) at the same time as it agrees with var. *elongata* Olney. He also mentions that *C. chlorostachys* is only the form of *C. capillaris* from forests at lower altitudes, whereas *C. Novograblenovii* is thought to correspond to *C. Williamsii* although perhaps differing from it in some minor characters. In addition to the main species, Hultén (149) reports *C. Williamsii* and *C. Krausei*, both of which were originally described from the area of his flora, and points out that the latter does not have any counterpart in eastern Asia.

A parallel of *C. Krausei* was, however, discovered by Polunin (288) in Greenland and Iceland and described as var. *Porsildiana* of *C. capillaris*. In

a preliminary treatment by Boivin (35), reporting the occurrence of this race also at the west coast of Hudson's Bay and at Cape Dalhousie on the arctic coast, this variety is regarded as equivalent to the var. *typica*, var. *minima*, and var. *Krausei*. And the typical race of *C. capillaris* is divided into f. *typica* and f. *major*, which is var. *elongata* Olney.

In a short but general treatment of the section *Capillares*, Raymond (300) deals mainly with the history and distribution of *C. Williamsii* but also discusses the other species and races. It is shown that since *C. koreana* Komarov is a later homonym of *C. coreana* Bailey, it must be replaced by the name *C. tenuiformis* Lév. & Vaniot as had also been demonstrated by Akiyama (2). The taxon *C. lenaeensis* is regarded as synonymous with *C. Ledebouriana*, while *C. Novograblenovii* is thought to be at least closely related to *C. Williamsii*. Raymond (300) recognizes three varieties of *C. capillaris*: var. *typica*, var. *Krausei*, and var. *Regeliana*, the last one, however, by a mistake. The var. *typica* includes three formae: f. *typica*, f. *minima*, and f. *major*, the last of which is identical with var. *elongata*. And the var. *Krausei* is shown to be so closely related to the var. *Porsildiana* that the latter is regarded only as a forma of the former (cf. also Raymond, 299). Although the status of these races is not in conformity with their biological levels as discovered by the present writers, there is no doubt that Raymond (300) is right in uniting the forms mentioned within these two taxa, as has already been confirmed by Löve and Löve (220).

In his book on the botany of southwestern Yukon adjacent to the Canol Road, Porsild (292) discusses the American representatives of the *Capillares* section. He takes the Linnaean *C. capillaris* in a very strict sense (cf. also 293), including only the typical arctic–alpine variety *tenuior* attributed to Drejer and the lowland variety *elongata* Olney. It is pointed out that although the latter is identical with f. *major* sensu Kükenthal, it is distinct from its basonym var. *major* attributed to Drejer and pictured in Flora Danica, which, in turn, is regarded to be very close to var. *Porsildiana* Polunin. *C. Krausei* is identified with var. *nana* Kük. and said to be known from a single station in the Yukon and a few localities in Alaska. On the other hand, *C. Williamsii* is listed only from its type locality at Dawson, although Raymond (300) had listed seven other localities within the area where Porsild himself had collected the species.

The taxon *C. neo-filipes* described by Nakai (251, 252) from Japan was reduced to a variety of *C. tenuiformis* by Ohwi (in 124), while Akiyama (4) regards it as a distinct though closely related species. The latter author also accepts f. *puberula* as a rare Japanese taxon of *C. tenuiformis*. The *C. selengensis* described by Ivanova (158) from Mongolia undoubtedly belongs to the tall-growing taxon of *C. capillaris*, while the taxon *C. Licentii* described by Nelmes (255) on the basis of a single specimen is certainly identical with the var. *parvirostris*, which Kükenthal (183) described under *C. capillaris* although it is more correctly placed under *C. Karoi*, from which it differs in some minor characters.

The most recent contribution to the understanding of the variability of the *Capillares* section is that by Böcher (29) discussing the Greenland variations. In typical *C. capillaris* he includes only var. *tenuior* and var. *minima*; the latter is supposed to be an arctic race, alpine in the subarctic regions but occurring down to sea level in the north. The terminal spikes of both these varieties are always staminate. It is shown that the Greenland race used as the basonym for the f. *major* attributed to Drejer by Kükenthal (181) and identified by him and others with the lowland type, var. *elongata* Olney, is, in fact, very different from the latter since its terminal spikes are gynoecandrous, or, very rarely, staminate. In this character, this tall-growing Greenland plant is similar to the taxa *Krausei* and *Porsildiana* regarded by Böcher (29) as good subspecies of *C. capillaris*, and he also proposes a subspecific status for this endemic Greenland taxon under the name of ssp. *robustior* (Lge.) Böcher. It is a continental-lowarctic plant, markedly calcicolous and oxyphobous (29, 30), which was originally depicted in Flora Danica (Tab. 2374, Fig. 3) and mentioned by Drejer (80) as "β major et robustior (subpedalis) spicis suboctonis, terminali androgyna (apice v. rarius basi feminea) feminei sub 10-floris, perigynis minoribus, pallidioribus longe stipitatis ovato-oblongis, caryops. concolore grisea". It seems to be as distinct as some of the Asiatic species, but was originally named as a variety β *robustior* by Lange (191), though hardly legitimately as will be mentioned later. The description by Drejer (80) and the picture in Flora Danica are the basis of f. *major* attributed to Drejer by many authors and wrongly identified with the plant treated as var. *elongata* Olney (cf. 88) by Kükenthal (181).

III. Material and Methods

The first cytological material from this section of *Carex* to be studied by the first two authors, who are responsible for the present cytological studies, was collected in the Torneträsk region of Lappland in northern Sweden during the summer of 1942, in the Östersund region of central Sweden the same year, and in Åhus in southern Sweden during the two following summers. The Lappland plants from the alpine localities were very small, with culms about the same length as the leaves and hardly reaching 3 cm. in height, and with short spikes having only two to five flowers each. This is f. *minima* of the typical race, and its additional characters are the curved culms, dark green or yellowish and usually much curved leaves which are folded and involute, and perigynia with a distinct and almost completely glabrous beak. The plants from the subalpine valleys in Lappland and from the Östersund region were taller: the straight culms about 10–15 cm. high with 5–10 flowers in each spike and always reaching well above the longest leaves. These latter plants belong to *C. capillaris* proper and are identical with the var. *tenuior* attributed to Drejer, and f. *typica* Boivin, but not with var. *major* Blytt typical of the lowlands farther south in Scandinavia. Both were collected in mires, while the plants from the Åhus region were taken from a mixed but mainly coniferous forest where they reached up to 40 cm. height. The culms

of the Åhus plants are straight with 10–20 flowers on each spike, and the plants are loosely tufted with 2–3 mm. broad leaves which always are distinctly shorter than the culms; the perigynia taper to a short and slightly dentate beak. The Åhus plants certainly belong to var. *major* Blytt and their identity with *C. chlorostachys* was realized by Kreczetovicz (177). They are certainly not identical with the plants from the northern valleys and are more than occasional forest ecotypes of the main species.

All the Swedish material studied cytologically, as well as all Scandinavian herbarium specimens seen by the writers, has only staminate terminal spikes. This bears out the observation by Hultén (194) and Böcher (29), who never saw any Scandinavian specimens of the *Capillares* section with gynoecandrous terminal spikes.

The first extra-Scandinavian material studied cytologically by the writers originated from several localities in all parts of Iceland, and was collected during the summers of 1946–50, from the highlands as well as the lowlands. All the lowland plants were of the same type as those from the subalpine valleys of Lappland in Sweden, i.e. they belong to the typical *C. capillaris*, and their terminal spikes are always strictly staminate. The highland types, however, belong to two distinct categories differing in the sexual condition of the terminal spikes and in some other essential characters. The more common highland taxon is identical with the Scandinavian alpine f. *minima*: small plants with culms no longer than the curved leaves, short and very few-flowered spikes, and clearly staminate terminal spikes. But small plants from the mountains between Eyjafjördur and Skagafjördur in the northern part of the country were found to have more erect culms, usually longer than the leaves, with 5–10 flowers in each spike which is denser even though the perigynia are smaller than in the other taxa. The beak of the perigynia is also very short and slightly dentate, but the most remarkable difference between these and other specimens from the Icelandic highlands is the terminal spike which is never completely staminate but usually clearly gynoecandrous with a variable number of female flowers. This is the plant described by Polunin (287, 288) as the var. *Porsildiana* of *C. capillaris* from southwestern Greenland, Iceland, and North America. According to Böcher (29), a Greenland collection of this race studied by him has gynoecandrous terminal spikes only in about 50% of the specimens. This is, however, not so in the Icelandic populations, although specimens with only one or two female flowers in the terminal spike have been observed. The mature perigynia fall off more easily from the terminal spike than from the completely pistillate spikes.

Since the transfer of the first two authors to the North American continent, a number of living plants of the *Capillares* section have been studied and fixed in the field. Most of these plants have come from the prairies of central Canada where the species *C. capillaris* is represented by the var. *elongata* in margins of wet forests; it is certainly the *C. saskatschewana* of Böckeler (32). In addition to this, seeds of recent herbarium specimens originating from

Alaska in the west to Greenland in the east have been secured and germinated and the chromosome numbers studied from the root tips of the young plants. Also, several taxa not met with on this continent but described from Asia have been grown from seeds of herbarium specimens collected at or not far from their type localities whenever available. In this way cytological material has been obtained of *C. capillaris* s.str. from Greenland, Gaspé, northern Manitoba, and northern Russia; of f. *minima* from Southampton Island and Chesterfield Inlet in the Northwest Territories; of var. *elongata* from Alaska and Newfoundland; of ssp. *robustior* from western Greenland; of *C. Krausei* from Alaska; of *C. Williamsii* from Ungava and Alaska; of *C. Sedakowii* from Ob and the neighborhood of Yakutsk; of *C. chlorostachys* from some localities in Siberia; of *C. tenuiformis* from the Udsk region of the Soviet Far East; of *C. Ledebouriana* and *C. Karoi* from the Altai region; of *C. Novograblenovii* from a couple of places in the Anadyr region; and of *C. Chamissonis* from central Okhotsk.

In this connection it should be mentioned that although in many genera it is possible to get good cytogenetical material by germinating seeds of herbarium specimens, this is not always easy with the genus *Carex*. Fresh seeds of this genus rarely germinate, as pointed out by Heilborn (128, 129), while after a resting period of 6–8 months the germination has risen to about 40–50%, but is again down to 1–2% after two years if kept at room temperature. It was discovered, however, that if the seeds are stored for several weeks prior to germination at about freezing they germinate almost completely 6–8 months after harvesting and at least 50% after 3 or 4 years' storage at room temperature. Also, in the case of herbarium specimens, seeds which did not germinate during two months in moist and sterile sand, sprouted very soon after storage in a refrigerator for a week or two. By aid of such a process it is even possible to get a low frequency of germination from some herbarium material of the *Capillares* section almost 20 years old, but no treatment could revive a single seed from older samples.

All the cytological studies so far made with the *Capillares* group by the present writers were based on root-tip material only. They do not share the experience by Davies (75) that reliable chromosome counts are difficult to obtain from somatic mitoses in *Carex*; on the contrary they find the meiotic stages to be more difficult and obscure in some of the species of other sections preliminary investigated because of complications from puzzling meiotic configurations and stickiness. Since it was found that not even the most refined techniques made any visible differences as to variations in chromosome morphology (cf. also 325), only the Svalöv modification of Navashin's fixative (cf. 222) was used for the chromosome plates depicted in the present paper. The roots were then embedded in paraffin, sectioned, and stained in crystal violet according to Suomalainen's (344) modification of Newton's classical method (cf. 221, 222).

IV. Cytological Observations

A. Previous Studies

The only chromosome number reported from a species of the *Capillares* section prior to the start of the present study is $n = 27$ counted by Heilborn (128) on meiotic material of *C. capillaris* from Skellefteå in northern Sweden. The drawing of the plate showed variation in the size of the chromosomes at meiosis, but the material was probably not fit for a detailed analysis since nothing was mentioned about the chromosome morphology of this taxon. This number was confirmed by Levan (in 214) on meiotic material from Lappland. The number $2n = 54$ was reported for Icelandic material of *C. capillaris*, and $2n = 36$ for Icelandic and American plants of *C. Krausei* ssp. *Porsildiana* and for American material of ssp. *Krausei* by Löve and Löve (220) studying root-tip divisions only.

B. Present Observations

The chromosome numbers and morphology were studied in detail in all the available material with the results reported below. The names used are those given above without any commitment as to the real taxonomical position of the different taxa. These will, however, be evaluated and co-ordinated on a later page. For the sake of convenience, the taxa with the lowest chromosome numbers will be discussed first, those with the highest ones last, although in that way the conventional order of the taxa is drastically broken.

1. Carex Williamsii Britton

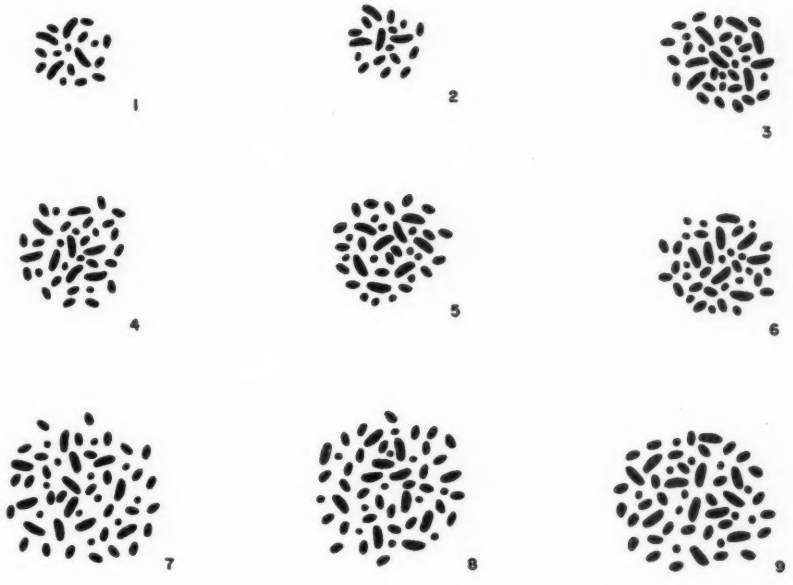
The somatic chromosome number of this species was found to be $2n = 18$ from Ungava (Fig. 1) as well as from Alaska. The chromosomes vary in size from about 0.5 to about 3.0 μ and no constrictions are observable. The chromosomes can be grouped into three classes based on length: four are long, or between 2 and 3 μ; 10 are medium sized, or between 1 and 1.5 μ; whereas four are very short, or only about 0.5 to 0.7 μ long and sometimes almost spherical. This same size variation was met with in all the cells studied from a dozen seed plants from both regions, and no more variability within the chromosome groups was observable between the populations from the east and the west than from within each of them, or even from different cells of the same individual.

2. Carex Novograblenovii Kamarov

The chromosome number $2n = 18$ could easily be counted on three seedlings from the Anadyr region, but an exact study of the morphology of the chromosomes could be performed in a few cells only (Fig. 2). There are four long chromosomes, 10 medium sized, and four short, or exactly the same number from each class as in *C. Williamsii*. It should also be mentioned that the variations within each class were the same as those observed within the American material of the latter species.

3. Carex Krausei Böckeler

The material of *C. Krausei* from Juneau in the Eastern Pacific District of Alaska was found to have $2n = 36$ chromosomes (Fig. 3) as determined in six



FIGS. 1–9. Mitotic chromosomes of *Carex* sect. *Capillares*. Magnification × *ca.* 2000.
FIG. 1. *C. Williamsii* Britton (Ungava), $2n = 18$.
FIG. 2. *C. Novograblenovii* Komarov (Anadyr), $2n = 18$.
FIG. 3. *C. Krausei* Böckeler (Alaska), $2n = 36$.
FIG. 4. *C. capillaris* L. var. *Porsildiana* Polunin (Iceland), $2n = 36$.
FIG. 5. *C. Chamissonis* Meinshausen (Okhotsk), $2n = 38$.
FIG. 6. *C. tenuiformis* Léveillé & Vaniot (Udsk), $2n = 40$.
FIG. 7. *C. capillaris* L. f. *typica* Boivin (Iceland), $2n = 54$.
FIG. 8. *C. capillaris* L. f. *minima* Beck (Lappland), $2n = 54$.
FIG. 9. *C. capillaris* L. var. *elongata* Olney (Manitoba), $2n = 54$.

seedlings. Also in this taxon it is not difficult to distinguish between three size classes of the chromosomes: eight long ones varying between 2 and 3 μ, 20 medium sized ones between 1 and 1.5 μ, and eight short ones between 0.5 and 0.7 μ. This number, based on the same material, has been previously published by Löve and Löve (220).

4. Carex capillaris L. var. *Porsildiana* Polunin

Nine plants from the mountains between Eyjafjördur and Skagafjördur in northern Iceland, mainly from above the farm Audbrekka in Hörgárdalur, were found to have $2n = 36$ chromosomes (Fig. 4). The same number was also counted on material grown from seeds originating at Fort Chimo in Ungava (three seedlings), from Chesterfield Inlet in the Northwest Territories (seven seedlings), from Frobisher Bay in Baffin Island (three seedlings), from Churchill in Manitoba (two seedlings), and from four seedlings at a couple of localities in northern Alaska. In all these collections not only the same chromosome number was observed, but also the same variation in size.

Every plate which could be properly analyzed was found to have eight long chromosomes, 20 medium sized, and eight short chromosomes, varying in size between 2 and 3 μ, 1 and 1.5 μ, and 0.5 and 0.7 μ, respectively.

5. Carex Chamissonis Meinshausen

The only material available of this rare eastern Asiatic species was a collection almost twenty years old from near the coast of central Okhotsk, as well as still older ones from some other localities in the Soviet Far East. Only one seed from the first-mentioned herbarium sheet germinated after prolonged cold treatment, but its mitotic divisions were numerous and it was easy to determine the chromosome number $2n = 38$ in several cells (Fig. 5). A detailed analysis of the morphology of the chromosomes revealed that eight were long, or between 2 and 3 μ, 18 were medium sized, or between 1 and 1.5 μ, while 12 were short, or only 0.5 to 0.7 μ long. Because fragmentation of chromosomes is known to be more frequent in aged seeds than in fresh material (cf. Tischler, 380, 381), and because germination was so low, it was deemed necessary to check very carefully the number of the medium and small chromosomes. These were found to differ in number from the taxa described above. But since all the cells were found to have the same distribution of the three size classes and none had a number deviating from $2n = 38$, it is not likely that the differences could have been caused by fragmentation of chromosomes in the seed during storage.

6. Carex tenuiformis Léveillé & Vaniot

A few seeds germinated from a specimen collected in the Udsk region of the Soviet Far East and correctly determined as *C. koreana* Komarov by the Russian collector. The chromosome number in three seedlings was found to be $2n = 40$ (Fig. 6). Of these chromosomes, eight were long, 16 were medium sized, and 16 were short. The same number and size variations were observed in several cells of all the seedlings.

7. Carex capillaris L. f. *typica* Boivin

Material of this taxon (more than fifty specimens from the valleys of Lappland and the Östersund region in Sweden, the Icelandic lowlands, southwestern Greenland, Gaspé, northern Manitoba, and northern Russia) was found to have $2n = 54$ chromosomes, as previously reported by Heilborn (128) and Levan (in 214) from Swedish material. A metaphase plate from an Icelandic plant is shown in Fig. 7. Despite the fact that many good plates were available for studies of the material from the other regions, no differences in the number or morphology of the chromosomes could be observed. In all the plates there were 12 long chromosomes, varying between 2 and 3 μ, 30 were medium sized, or between 1 and 1.5 μ long, and 12 were short, or only 0.5 to 0.7 μ long. Since all the size classes here are three times more numerous than in the taxa with $2n = 18$ chromosomes, it seems evident that this taxon is a hexaploid of the basic number $x = 9$, which is typical of the diploids *C. Williamsii* and *C. Novograblenovii*.

8. Carex capillaris L. f. *minima* Beck

The Scandinavian, Icelandic, and American specimens of this arctic–alpine ecotype (25 plants) were all found to have $2n = 54$ chromosomes (Fig. 8), and no differences in the size classes of these chromosomes could be found when compared with f. *typica*. All the cells have 12 long, 30 medium, and 12 short chromosomes.

9. Carex capillaris L. var. *elongata* Olney

This forest race from the prairies and lowlands of northern North America, ranging from Newfoundland in the east to Alaska in the west, has $2n = 54$ chromosomes (Fig. 9). Of these, 12 are long, 30 are medium sized, and 12 are small, and the variation within each of these size classes seems to be exactly the same as in the other 54-chromosome taxa described above.

10. Carex chlorostachys Steven

Specimens more than ten years old were obtained from three localities in central Siberia. Only a few seeds germinated and although the chromosome number could be counted in them all, just a few originating from the Tomsk region gave plates satisfactory for a detailed morphological analysis of the chromosomes. However, not more than one metaphase plate from each of three seedlings could be exactly analyzed. The number is $2n = 54$ (Fig. 10), and of these there are 12 long, 12 short, and 30 medium sized chromosomes.

The same number and size variation are typical also for the seven plants of the forest race of *C. capillaris* s.lat. from Åhus in Sweden (Fig. 11), which, according to the classification by Kreczetovicz (177), would undoubtedly belong to this taxon.

11. Carex capillaris L. ssp. *robustior* (Lge.) Böcher

The material of this taxon originated from the central and inner parts of Söndre Strömfjord in western Greenland. Rather good germination was obtained since the specimens had been collected recently, and five plants could be studied in detail. They were all found to have $2n = 56$ chromosomes (Fig. 12). The variation in size was the same as in the other taxa, i.e. the long chromosomes were between 2 and 3 μ long, the medium sized ones were 1 to 1.5 μ, and the short ones 0.5 to 0.7 μ. While 12 chromosomes in the plates studied always belonged to the long class, only 28 were medium sized and as many as 16 belonged to the shortest group.

12. Carex Sedakowii C. A. Meyer

This rather delicate species with gynoecandrous terminal spikes was represented in the cytological material by specimens originating from Ob and the neighborhood of Yakutsk. Only a few seeds from each locality germinated, and detailed studies on the morphology of the chromosomes could be made on only a few plates from each sample. The chromosome number was found to be $2n = 56$ (Fig. 13) with 12 belonging to the class of long chromosomes, whereas 28 were median and 16 short. Thus, no differences in the size or number of the chromosomes could be discovered between this species and the last-mentioned taxon, even though they differ in morphology and are very widely separated in area.

FIGS. 10–15. Mitotic chromosomes of *Carex* sect. *Capillares*. Magnification × *ca.* 2000.
FIG. 10. *C. chlorostachys* Steven (Tomsk), $2n = 54$.
FIG. 11. *C. chlorostachys* Steven (Sweden), $2n = 54$.
FIG. 12. *C. capillaris* L. ssp. *robustior* (Lge.) Böcher (Greenland), $2n = 56$.
FIG. 13. *C. Sedakowii* C. A. Meyer (Yakutsk), $2n = 56$.
FIG. 14. *C. Ledebouriana* C. A. Meyer (Altai), $2n = 56$.
FIG. 15. *C. Karoi* (Freyn) Freyn (Altai), $2n = 58$.

13. Carex Ledebouriana C. A. Meyer

Only two seeds of an old Altai collection of this Siberian species germinated after prolonged cold treatment. The chromosome number counted in several plates from both seedlings was found to be $2n = 56$ (Fig. 14), and the set was found to include 10 long ones, 34 medium sized, and 12 short chomosomes.

14. Carex Karoi (Freyn) Freyn

This is a delicate and rather tall-growing Siberian and eastern Asiatic plant, recalling somewhat the forest race of *C. capillaris* from North America, although morphologically clearly distinct from it. Eight seedlings were grown from recent Altai material, and their chromosome number was found to be $2n = 58$ (Fig. 15). The idiogram was made up of only 10 long chromosomes, as many as 32 medium sized ones, and 16 short units.

V. General Observations and Discussion

Although less than one-tenth of the already described species of higher plants have been studied by cytologists, it has become evident that the evolution of sterility barriers and, naturally, of species has ensued two main lines, one of which has followed at least three more or less different cytological patterns. The majority of taxa seem to have evolved mainly by a gradual differentiation and repatterning within and between the same chromosome set, without any alteration in the number of chromosomes. This gradual speciation is met with in almost all genera, but nowhere is it as

dominant as in the family Umbelliferae or in the subdivision Gymnospermae, both of which are almost exclusively characterized by genera devoid of polyploid chromosome numbers. The other main group of speciation is characterized by the so-called abrupt evolution (cf. 155, 209, 213, 390). The abrupt formation of sterility barriers is usually connected with numerical changes in the chromosome set. Such changes are most frequently euploid, so that the different chromosome numbers are even multiples of the same basic number. This euploid series may be either autoploid, i.e., a multiple of the same original and almost undifferentiated set, or alloploid, i.e., based on the addition of diploid sets which have originated by differentiation from the same original complement (cf. 72, 160, 213, 247, 334, 380, 381). Some times the euploid series may be based on the so-called agmatoploidy, i.e., transverse division of all the chromosomes of a set, best studied in the genus *Luzula* (cf. 259, 261). Numerical variations of an aneuploid or dysploid kind, based on an original loss or gain of extra chromosomes fewer than are in a full set typical of the basic number of the taxon and its relatives, were first demonstrated as specific differences in *Erophila* (403, 404), and have since been found to be typical for several other groups (cf. 378, 381). These latter variations are most often found to be caused by stabilization of supernumeraries or by the occurrence of so-called B-chromosomes which are mainly heterochromatic. The former type is perhaps best known in a stabilized form in the genera *Crepis* (cf. 9, 10, 334) and *Iris* (cf. 297, 328, 379), whereas the latter has recently been studied in great detail in several genera, mainly of Monocotyledons (for references, cf. 38, 72, 248, 381). The stabilization of supernumeraries has certainly been of great evolutionary significance, while the taxonomical importance of the B-chromosomes still seems disputable. Finally, there is the dysploid type of variation originally reported from the genus *Carex* by Heilborn (127, 128), characterized by different but only rarely polyploid numbers occupying practically all even multiples from $2n = 12$ to $2n = 112$.

Although the variations in euploidy have been thoroughly studied so that they are now satisfactorily understood, those based on stabilization of supernumeraries or B-chromosomes are still not completely explained. It is known, however, that the supernumeraries are ordinary chromosomes which behave in the normal way and are structurally inseparable from these; the B-chromosomes may differ in size and vary in number without a marked genetic effect since they seem to be mainly heterochromatic though perhaps not genetically completely inert. The latter are often recognizable by their precocity or by clear differences in localization of chiasmata at meiosis. An increase in number of chromosomes through euploidy or aneuploidy connected with supernumeraries or B-chromosomes is always followed by an increase in the total length of the chromosome set and an increase in chromatin matter. It has already been observed, however, by Heilborn (128) and confirmed by all later authors, that a dysploid increase in chromosome number in the genus *Carex* usually is followed by a decrease in number of long chromosomes and

not necessarily by an addition in total length or in the total amount of chromatin. This phenomenon persuaded Heilborn (128) that the main cause of increase in chromosome number and alterations of basic numbers in this genus must be a transverse division or fragmentation of the chromosomes, including some ploidy variations also. Since this seemed to contradict all the well-founded theories of chromosome division based on innumerable observations of cell divisions of other plants and animals, Heilborn (135) and Wahl (395) tried to eliminate the hypothesis of fragmentation as a mode of increasing the number of chromosomes in *Carex*, even though they were not able to replace it with a more satisfactory general explanation.

It is a well-known fact that each chromosome is characterized by a constriction zone, termed centromere or kinetochore, which is of vital importance for cell divisions. Occasionally chromosomes are found to possess two such constrictions, which then disturb the normal cell division (281, 284, 294, 307, 392, 398, 412). When a chromosome is broken, naturally or experimentally, only fragments with a centromere will be able to survive many cell divisions while acentric fragments soon disappear. In *Carex*, however, it was shown by Heilborn (128) that the chromosomes of this genus usually are without a visible constriction, and all later students have been able to verify this observation, although the explanation was ventured that this could be due to the small size of the chromosomes combined with an unsuitable fixing method. But even the most delicate methods for fixing the centromere zone still do not result in any such differentiation in this or closely related genera (325).

The genus *Carex* is not appropriate cytological material, and its small chromosomes make it especially unsuitable for studies on the activities of the kinetochore during cell division. Lately, however, more suitable material has been found in the related genus *Luzula*. Studies on the cytology of *L. purpurea*, which has only six very large chromosomes in the somatic phase, revealed that the chromosomes of this species have no centromeric constriction. The constriction zone is here replaced by a kinetochore activity extended all over the chromosomes. Species of *Luzula* with $2n = 12$ were found to have chromosomes approximatively half as large as the 6-chromosome species, and those with a higher number may have still smaller ones. It was therefore suggested that, because of the non-localized kinetochore, fragments would be able to survive in this genus (cf. 16, 21, 28, 43, 44, 49, 90, 97, 229–234, 245, 258–261, 266, 267). The correctness of this hypothesis seems to have been verified by aid of X-ray experiments (cf. 45, 46, 51, 52, 188, 260), although the occurrence of a diffuse or polycentric kinetochore or fragmentation has been questioned by some authors (284, 305, 377, 381). There is, however, almost complete agreement as to the occurrence of the extended kinetochore and the subsequent survival of fragments, although some disagreement still exists as to the real nature of this kind of kinetochore. The disagreement is, however, mainly in interpretation, and it can be expressed in the differences in naming the phenomenon. The majority of the *Luzula* workers, and with them many others studying similar kinetochores in some lower animals and

plants, speak about the phenomenon as the diffuse kinetochore or centromere (cf. 15–18, 44–46, 48–57, 97, 110, 142, 161, 228–234, 243, 245, 258–261, 265–267, 280). Others term it polycentric or compound constriction or kinetochore (cf. 39, 72, 103, 188, 306). Outside the genus *Luzula* a similar condition was postulated for some Conjugatae, desmids, and Plasmodiophorales as well as for some nematodes (98, 99, 103, 104, 105, 145, 147, 320, 401); Chakravorti (58–63) indicates the occurrence of at least related phenomena in the Zingiberaceae and Musaceae. Such a kinetochore has been definitely demonstrated for several insects (cf. 18, 146, 321, 343), and for *Eleocharis* (15, 110), *Scirpus* s.str. and related genera (325), and *Juncus* (Löve and Löve (215 and unpubl.)), although *Eleocharis* and *Scirpus* show some variations from the typical conditions in *Luzula*. Approximately the same variations are encountered in *Carex* (cf. 75), whereas the related genus *Fimbristylis* is characterized by one distinct centromere only on each chromosome (325).

The appearance and action of the chromosomes during mitosis is very different in taxa with a distinct centromere and in those with a diffuse or polycentric kinetochore. The centromeric constriction typical of the chromosomes of the higher plants is clearly visible, even after bad fixation, and it lies distinctly in the equatorial plane at metaphase while the chromosomes themselves bend in all directions. In plants with a diffuse or polycentric kinetochore, however, no such constriction is ever visible, and the chromosomes bend only in the equatorial plane, never towards the pole during metaphase. Chromosomes with a distinct constriction zone always move with the centromere ahead of the ends at anaphase, forming the typically concave anaphase nucleus, while those with a diffuse or polycentric kinetochore separate either quite parallel or the ends start the division so that the anaphase group sometimes may become very convex. The meiotic divisions, too, are somewhat different from the normal ones in that a certain kind of stickiness is rather common (48, 47, 243), and it has been postulated, not without evidence, that they might be characterized by a cytological postreduction rather than by the typical prereduction. This is, however, not quite clear yet since many of the reports lack either clarity or definition of the terms used, or both (cf. 18, 272, 273, 274, 332, 333, 389, 393).

As can be seen from the figures of the mitotic chromosomes in the present paper, all the taxa of the *Capillares* section have only chromosomes with the non-constrictional appearance typical for plants with a diffuse or polycentric kinetochore. In side view of the metaphases even the largest of the chromosomes were always found to be kept exactly in the equatorial plane, and from a polar view all the chromosomes are in the plane at exactly the same level which would make drawing and measuring easy if it were not for their small size. At anaphase, the parallel separation of the chromosomes peculiar to *Luzula* is typical of the smaller units, whereas the larger chromosomes start the separation at the ends. All the chromosomes move faster at the ends than at their middle parts so that at late anaphase the group is often distinctly convex. These observations all bear out the hypothesis that the chromosomes

of *Carex* do not possess a single centromere but are instead characterized by a diffuse or polycentric kinetochore of much the same kind as studied in detail from the genus *Luzula*. The same conclusion was reached by Davies (75) from her particularized studies in the *Carex flava* group.

The occurrence of a polycentric or diffuse kinetochore renders feasible the survival of more than one fragment of each chromosome and, thus, an increase in number without an increase in total length of the chromosome set. Such a contingency must be accepted as certainly demonstrated in the genus *Luzula* where usually all the chromosomes of the set seem to divide simultaneously to double the chromosome number, although real polyploidy also is met with and occasionally only some of the chromosomes have been fragmented. This phenomenon is what Malheiros-Gardé and Gardé (233) very appropriately termed agmatoploidy (= fragment-ploidy), while Battaglia (17) proposed for it the term pseudopolyploidy or even agmatopseudopolyploidy. The terminological discussion by Battaglia (17) is of interest since it shows that the solution of the problem is not so easy as it may look and several phenomena related to this one may not be similar to it. But his criticism of the term agmatoploidy because of its Greek origin also hits the equally Greek origin of the terms polyploidy and pseudopolyploidy. Irrespective of the original coining of the word polyploidy from the Greek prefix *polyplos* and Weismann's (400) *id* (cf. 341, 342, 405), it is usually shortened to ploidy only by recent cytologists (cf. 72) when they are discussing numerical variations without a closer definition of their real nature. If the word ploidy is defined as some change in the chromosome number, its subdivisions would logically be polyploidy (autoploidy, alloploidy, amphiploidy) and aneuploidy or dysploidy when all or some of the chromosomes are multiplied in the normal way, and agmatoploidy when the numerical additions are caused by a transverse division of chromosomes with a diffuse or polycentric kinetochore. Like polyploidy, agmatoploidy may be complete, involving a transverse division of all the chromosomes in a set, or it may be partial, involving some of the chromosomes only, and thus comparable to dysploidy or aneuploidy in the former group, and it may or may not be connected with real polyploidy.

Complete agmatoploidy is typical for *Luzula* (cf. 259, 261), although partial agmatoploidy occurs (cf. 90, 259, 261, 329), and both may be combined with ordinary polyploidy. In *Carex*, however, Heilborn (128) concluded that the transverse division of the chromosomes never could be in whole sets simultaneously, but in single or few chromosomes only. This indication of partial agmatoploidy was based on the fact that there are at least three distinct size classes of chromosomes encountered in the genus, and all may be found within the same cell. The largest chromosomes, however, are most frequent in species with low numbers, while species characterized by a high number of chromosomes usually have few or no large chromosomes left. In addition to fragmentation, other changes in basic number were supposed to be caused by duplication of single chromosomes, by polyploidy, and by additional phenomena known to occur in other groups of plants.

At present about 300 species of *Carex* have been studied by cytologists in several regions. The chromosome numbers range from $2n = 12$ to $2n = 112$. It seems evident from observations within some sections that polyploidy occurs as frequently as does agmatoploidy, although partial agmatoploidy sometimes seems to have followed the polyploid changes so that these are no longer distinguishable as direct multiples of a common basic number. At present not a single example of complete agmatoploidy within a section is known, while cases of polyploidy even without major modifications by later partial agmatoploidy are known from within several sections. Unmodified polyploidy is reported from *C. siderosticta* Hance s.lat. with $2n = 12$ and 24 chromosomes (357), *C. stenantha* Franch. & Sav. s.lat. with $2n = 34$ and 68 chromosomes (373), *C. humilis* Leyss. s. latiss. with $2n = 36$ in England and $2n = 72$ in Japan (75, 368, 372), *C. saxatilis* L. and *C. miliaris* Michx. with $2n = 80$ and 40 respectively (212), *C. salina* Wg. and *C. subspathacea* Wormskj. with $2n = 84$ and 42 (220), and it is indicated though modified in *C. Buxbaumii* Wg. and *C. adelostoma* Krecz. with $2n = 74$ and 106 chromosomes (196), and in several other groups. Although some of these examples may seem to indicate the occurrence of the so-called intraspecific polyploidy, this is probably not so but is due only to lack of taxonomical studies on the material. There is no doubt that ordinary polyploidy occurs also in the *C. multifolia* group with $2n = 30$ and 60 chromosomes, although the latter multiple is further complicated by the occurrence of individuals with $2n = 62$, 64, 65, 66, and 70 chromosomes formed by secondary partial agmatoploidy (cf. 373). A similar situation occurs within some of the other sections such as *Frigidae*, *Montanae*, *Multiflorae*, and *Careyanae* (cf. 373). As pointed out recently by Tanaka (373), secondarily balanced polyploids are more frequent than are euploids in many sections, and there is little doubt that additional partial agmatoploidy or even deletion of chromosome pairs plays prominent role in modifying the euploid numbers. Related species are often characterized by like numbers and similar frequencies of size classes of the chromosomes whereas sections differ more widely in basic numbers and, especially, in the frequencies of the three main size classes of the chromosomes.

Although Heilborn (128) was able to demonstrate that geographically widely separated populations of *Carex* species are always characterized by the same chromosome number, this opinion was not shared by Wahl (395) and Tanaka (373). The latter author even claims that at present varying numbers of from two to as many as six have been found in some seventy species, and that various degrees of structural hybridity have been noted in over fifty species, while only 59 are recognized as stable. Though a few of these numerical variations certainly are real, having been reported by an observer skilled in studies of just this genus, it seems equally safe to conclude that their great majority is only fictitious since different numbers are reported by different observers and probably are caused by difficulties in counting meiotic figures in structural hybrids, or even by lack of cytological skill by the occasional observer, bad techniques, or, perhaps mainly, wrong identification

of the material (cf. 381). Despite of Wahl's (395) and Tanaka's (373) criticism, the present writers are inclined to accept Heilborn's (128) statement on the stability of the chromosome number within the species of this taxonomically critical genus, although not completely rejecting the possibilities of the occurrence of small numerical variations within some species with high numbers, a fact also typical of practically every high-polyploid species of genera characterized by euploid multiples only.

The basic number of chromosomes in *Carex* is of certain interest from the point of view of the evolution of the genus. Heilborn (135), who knew the chromosome number of 73 species only, showed that although dysploid numbers are characteristic of the genus, the frequency peaks of the numbers known to him were at $2n = 56$, 84, and 112. On the basis of this he concluded that $x = 7$ must be the original basic number of the genus. Wahl (395) pointed out, however, that the additional data available to him had shifted the frequency maxima to 54, 68, 82, and 112, with numerous irregularities in between. He postulated that this might be caused by a loss of chromosomes and a secondary balance slightly below the real euploid number. Although he was of the opinion that the basic number $x = 7$ still should be regarded as the most typical one for the genus, he also pointed out that series based on $x = 5$, 6, or even 8 are not infrequent. This conclusion is still valid for the frequency peaks even though several new numbers have been added since the publication of the very detailed paper by Wahl (395); his diagrams remain sound for numerical variations within the genus (cf. also 373).

Although the considerations of the primary basic number in *Carex* and their later alterations were based partially on the hypothesis of the occurrence of fragmentation in the genus, other phenomena were supposed to be more prominent in the formation of the variations observed. The weight of such considerations based on frequency of certain multiples must, however, diminish considerably through the discovery of diffuse or polycentric kinetochores and partial or complete agmatoploidy within the group. Only in species groups having no increase in number without an accompanying increase in total length can the frequency of multiples be of any interest, except where it is possible to reduce the agmatoploid increases to the original number of unfragmented primary chromosomes. This is apparently possible not only within the *Capillares* section but also within the *C. siderosticta* complex which has a diploid number as low as $2n = 12$.

It is evident from the results described above from the *Capillares* section that its species belong to a polyploid series based on the secondary basic number $x = 9$, with some smaller irregularities at the polyploid levels. The lowest chromosome number is the diploid one ($2n = 18$), two taxa are exactly tetraploid with $2n = 36$, and four taxa exactly hexaploid with $2n = 54$, while the exceptions at the tetraploid level are one taxon each with $2n = 38$ and 40 respectively, and those at the hexaploid level showing three taxa with $2n = 56$ and one with $2n = 58$ chromosomes. There are clear indications that the deviating numbers are secondary and that they have originated by fragmentation after the tetraploid or hexaploid stages had been reached, since the

frequency of long and medium chromosomes has decreased in these deviating taxa at the same time as the frequency of their small chromosomes has increased proportionally. Thus, while the taxa with $2n = 36$ chromosomes have eight long, twenty medium, and eight short chromosomes, or exactly twice the karyotype of the diploids, the plants with $2n = 38$ chromosomes have lost two medium sized ones and gained four short ones; still two more medium sized chromosomes have been replaced by four short ones in the 40-chromosome taxon. The same occurs in two of the taxa with $2n = 56$, while the third taxon with that number has replaced a pair of long chromosomes with two pairs of medium sized ones. The species with $2n = 58$ seems to have lost two of its long chromosomes and added two medium sized and four short ones as compared to the taxa with exactly the hexaploid chromosome number. Even this latter deviation is easily explainable by partial agmatoploidy, assuming that the lost pair of long chromosomes has given rise to two pairs of medium sized ones at the same time as a pair of the latter group has fragmented into four short chromosomes. When taxa characterized by the same secondary number inhabit areas close to each other and do not show very great morphological differences, it may be postulated that they have originated from one such partially agmatoploid change only, followed later by gradual morphological differentiation. But when they are encountered in as widely separated regions as Greenland and eastern Asia and are morphologically not very closely related (as is the case with two of the 56-chromosome taxa characterized by the same number of chromosomes in each size class), it is most likely that the chromosomes involved in the secondary change have not been identical. To verify this assumption hybridization experiments are needed.

If the deviations from the euploid numbers in the *Capillares* section have been caused by partial agmatoploidy as just suggested, it is but logical to suppose that the same phenomenon has been the cause of the formation of the medium and short chromosomes of the basic set for the section. Thus, the four short chromosomes of the diploid could have derived from two subsequent transverse divisions of one long chromosome since their total length is approximately the same as that of the long units. Also, each of the medium sized units is about half the length of the long ones, so that the 10 medium sized chromosomes may have been formed by a transverse division of five long units. Since this would make six long chromosomes in addition to the four typical of the diploid set of section *Capillares*, the original diploid set before partial agmatoploidy took place would have been $2n = 10$ chromosomes, and the basic set for the genus thus could be postulated as $x = 5$. If this is correct, it would mean that the lowest diploid number to be expected within the genus *Carex* and its relatives should be $2n = 10$. Although species with $2n = 20$ may be pure tetraploids and must be so if all their chromosomes are of the long type, species with up to $2n = 40$ could as well be diploid from the point of view of chromosome length only, being at the same time completely agmatoploid with every primary chromosome fragmented into four short

units. In addition, although the primary basic number may be considered as $x = 5$, the occurrence of partial or complete agmatoploidy has modified this number so that for the purpose of verifying polyploidy karyotypical studies must be made to determine if a taxon with up to $2n = 40$ chromosomes is a polyploid or only an agmatoploid diploid. The secondary basic numbers formed by aid of agmatoploidy would vary between $x = 5$ and $x = 20$ if it is assumed that no more than four short chromosomes can be formed from one long one, but most of the secondary basic numbers will probably be at the lower and intermediate levels because of the dominance of partial agmatoploidy only.

Support for the hypothesis of $x = 5$ as the primary basic number of the genus *Carex* can be drawn also from another group of the genus studied in great detail by Tanaka (357): the diploid *C. siderosticta* with only $2n = 12$ chromosomes. According to Tanaka (357) the chromosomes measured during the pollen divisions could be divided into three classes according to their length. Two chromosomes were found to be 10.88 ± 0.23 units, two were 9.03 ± 0.15 units, while two were only 6.03 ± 0.18 units, each unit being $3.3\ \mu$. In the terminology used in the present paper, four of the chromosomes of the haploid set would fall within the class of long chromosomes, while two undoubtedly belong to the medium class. Assuming that the latter have originated from transverse division of a long chromosome, the primary basic number of the taxon which originally gave rise to the diploid *C. siderosticta* becomes $x = 5$, or exactly the same as arrived at through the same hypothesis when applied to the diploid taxon of the *Capillares* section.

In this connection it should perhaps be emphasized that since modern caricologists consider species with wide leaves and/or lateral fruiting-stems as primitive, the *Capillares* section must be regarded as rather advanced. *Carex siderosticta* and other species of the *Careyanae* section are, however, certainly primitive, so it is of special interest to find the lowest chromosome number so far counted within the genus from this group, even though much higher numbers have been counted in related species. It would certainly be worth while to make similar detailed cytological studies on primitive North American species of section *Careyanae* such as *C. plantaginea* Lam. or *C. platyphylla* Carey, as well as on their eastern Asiatic relatives close to *C. siderosticta* Hance: *C. pachygyna* Franch. & Sav., *C. exerta* Chu, *C. glossostigma* Hand.-Mazz., or *C. grandiligulata* Kük. Cytological studies are also needed on representatives of the primitive sections *Euprepes* Nelmes Airy-Shaw, *Hypolytroides* Nelmes, and *Mapaniifoliae* Nelmes & Airy-Shaw, which, unfortunately, are known mainly from poor herbarium material. And from the point of view of studies on the basic number and its development, great expectations may be anticipated from species such as *C. scaposa* C. B. Clarke of Indochina and southern China, *C. Adrienii* Camus, which is an endemic from Indochina with elliptical leaves up to 6 cm. broad and nearly 100 cm. long, and *C. Kucyniakii* Raymond from northern Tonkin, with a single persistent broad leaf and a lateral stem bearing few-flowered spikes and infundibuliform bracts (cf. 175, 256, 303).

Additional support for the hypothesis of $x = 5$ as the primary basic number for *Carex* in particular and the Cyperaceae in general may be drawn from the fact that this is the lowest haploid number observed in other genera of the family. The chromosome number $2n = 10$ has been reported for *Eleocharis parvula*, *Bulbostylis barbata*, and six species of *Fimbristylis*; several other species of these genera are polyploid based on this number. It should also be mentioned that although the occurrence of a diffuse or polycentric kinetochore seems to be universal in *Eleocharis*, as it certainly is in *Carex*, the genus *Bulbostylis* appears to be characterized by having chromosomes with such a kinetochore as well as others with a distinct constriction zone in the same cell. This is also indicated for at least some species of *Cyperus* s.lat. (325, Löve and Löve, unpubl.). But the genus *Fimbristylis* s.str. does not have any chromosomes with a diffuse or polycentric kinetochore and, consequently, no agmatoploidy or dysploidy caused by partial agmatoploidy is found in that genus (325). The fact that localized and diffuse or polycentric kinetochores may characterize not only a single species or genus but also different genera of the same family seems to be in accord with the conclusion by Lima-da-Faria (202) that both these types of kinetochores are to be considered as differentiated derivatives of the same process, not as representatives of phylogenetically sharply distinct lines of evolution. It is, however, equally logical to conclude that within the group of the Glumiflorae s.lat. the diffuse or polycentric kinetochore is a primitive feature typical of all genera of the Juncaceae, the most primitive member of the group from the morphological and anatomical point of view as well. The most primitive taxon of this family is the genus *Luzula*, as revealed by its morphological characters, and its primary basic number is $x = 3$. From this number is derived the number $x = 5$ typical of *Juncus*, which is characterized further by complete agmatoploidy and polyploidy, even though partial agmatoploidy is known from some species [cf. Löve and Löve (215, and unpubl.)]. Although agmatoploidy is typical of the genus, there are evidences indicating that its basic number has been produced by duplication rather than by agmatoploidy. From this number the $x = 5$ supposed to be typical of the primitive Cyperaceae could have derived. Within the Cyperaceae, however, a certain differentiation of the originally diffuse or polycentric kinetochore of the basic set has taken place so that some of its genera are still characterized by such a kinetochore while others have evolved a strictly localized centromere. Furthermore, the agmatoploidy in this family is rarely complete, whereas polyploidy and partial agmatoploidy are typical of most of the genera. It is feasible, therefore, that the primitive Gramineae with $x = 5$ may also have evolved from the primitive Cyperaceae with a localized centromere. The discovery of $x = 4$ as the basic number for some very primitive taxa of the grasses (23, 125, 205, 206, 242, 387) may, however, indicate that the solution is more complicated and that the Gramineae and Cyperaceae might as well be separate lines of evolution from a common ancestor, e.g. the Juncaceae. All this is, however, only a speculation and not even a hypothesis, although it is in line

with the studies on the embryology of these families (411) and their classification, based on morphology and anatomy, by Hutchinson (154) and Takhtajan (346).

VI. Taxonomical Conclusions

The present writers feel that before any conclusions as to the taxonomical position of the taxa of the *Capillares* section can be made on the basis of the cytological studies reported above, the categories in question must be clearly defined. Though an understanding of the significance of the different characters in a genus is essential for its classification, the biological units cannot be properly systematized without previous scientific definition. It is a general fallacy to attempt such a circumscription from the customary practice of recognized taxonomists instead of basing it on a characteristic common to all biota belonging to the same category from the biological point of view. The practice of distinguishing the units by degree of morphological differences only, or by discontinuities in several of their characters, is often purely arbitrary even though unavoidable sometimes because of lack of experimental evidence. Certainly the various processes of speciation acting in the vegetable kingdom have played diverse roles in different genera or even in different groups of the same genus (213). The development of morphological and physiological differences appears to be as multitudinous as are the species and genera themselves so that numerous definitions may seem to fit for various taxa and make impossible a single definition of each concept. This is, however, a common paralogism when the phenomena of evolutionary importance are not separated from those of significance only for recognition of the ultimate results of the evolutionary divergence. In fact, one single definition of the category of species fits all organisms despite differences in morphological and physiological variability and their evolutionary causes (cf. 192), although its practical execution may sometimes be more tedious than is desirable for ordinary taxonomical work. This is the definition hinted at already by Rajus (296) in the preface to his "Historia plantarum", where he clearly expresses that a species should be characterized first and foremost by "distincta propagatio ex semine". This limitation by sterility barriers was certainly followed by Linnaeus in his fundamental studies of the Swedish flora although perhaps not so strictly in his naming of foreign plants known to him only superficially through books or a few herbarium specimens. It was also followed by most later taxonomists, who used certain kinds of discontinuous variation to disclose this barrier with such a competence that even modern experiments cannot improve most of their conclusions. In modern words this concept has perhaps never been more clearly set forth than by Mayr (239) defining the *species* as "groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups." Potentially, though perhaps not always actually, interbreeding taxa consequently must be intraspecific variations only, classified as *subspecies* if they are morphologically and physiologically a more or less regional group of

populations of the basic taxon; as a *variety* if they are more or less local races; and as a *forma* when they are sporadic variations only (cf. DuRietz (82), Löve and Löve (214)). As a necessary result, taxa differing in chromosome number are to be regarded as distinct species (cf. 209–214, 219), whereas interfertile taxa are always conspecific, even when they are ecologically or geographically separate and differ in very conspicuous morphological characters: e.g., the *rubrum* and *album* races of *Melandrium dioecum* (223).

It is now known that the reproductive barriers between species can be produced in different ways and, accordingly, the morphological characteristics of use to the herbarium botanist will not always be comparable. A sudden and abrupt change in chromosome number or constitution is one such alteration—excepting numerical changes caused by B-chromosomes—and it is immediately followed by certain morphological and physiological alterations and later succeeded by further diversification. Most frequently, however, these barriers and other characteristics of the species are formed gradually by the response of the potentially more or less isolated populations to different selective environments (cf. 67, 68, 70, 71) or through gene mutation and genetic drift. In this latter case, the diverging taxa develop distinguishing traits, any of which may serve as diagnostic, and, starting from the local population, their evolution passes the stages of the ecotype and the forma, the variety, and the subspecies before the status of reproductively isolated species is reached (cf. 67, 68, 334, 390). This is an endless process; as soon as one taxon becomes reproductively isolated from another, populations of each will diverge and evolve gradually until the next barrier has been produced between them.

Although both these basic processes of evolution of sterility barriers result in species reproductively isolated from their relatives, the evolution of morphological characteristics useful for classificatory purposes is basically different for each group. In fact, gradual species and their intraspecific taxa may in most cases be distinguished by the degree of morphological differences, and biometric studies on herbarium material may give quite adequate indications as to when the sterility barrier had become sufficiently strong for the reproductive isolation required between sympatric species. The abrupt species, however, cannot always be separated by aid of the same methods, since the reproductively strongly isolated units may differ in fewer and less conspicuous characters than do their gradually developing intraspecific races. Detailed studies performed on a high number of abrupt species formed by auto- or hemi-alloploidy (cf. 218) have shown that diploids and polyploids, and polyploids at different levels, are always distinguishable by aid of floral and seed characters, differences affected by cell-size, leaf-form variations, hairiness variability, and several other characters which may escape the eye of a discriminating taxonomist used to looking for more conspicuous differences in degree as compared with lower units. This is true not only of alloploids, which usually combine characters of two species, but also of natural hemiautoploids and even recently produced experimental panautoploids. Not only conspicuous

characters previously used by herbarium taxonomists are of taxonomical importance but also every characteristic useful for the separation in the herbarium of the natural species isolated by a reproductive barrier.

It is pointed out that the above considerations are based on many genera with euploid chromosome number variations and strictly localized centromeres. Heilborn (128, 135) has shown that also in *Carex* differences even in only a pair or two of chromosomes are effective as reproductive barriers, and the same has been emphasized by almost all later students of this genus. It is evident, from the genus *Luzula* as well, that agmatoploid variations may be the initial cause of the reproductive isolation between species so that the originally broken chromosomes will soon deviate from their unbroken partners and form a barrier as strong as that formed by ordinary polyploidy between the new taxa.

It must be emphasized in this connection that whereas the great majority of species so far studied from the genus *Carex* is characterized by a very constant chromosome number, slight variation in number and size is typical of others (75). These latter species show no greater variability in morphology, and it is reasonable to suggest that these cytological variations are caused by meiotic irregularities not affecting their sterility mechanisms substantially, or even by some kind of B-chromosomes without visible genetic effects. In sections characterized by such variations small differences in chromosome number cannot always be indicative of specific barriers between the respective taxa. In sections without such intraspecific variations in chromosome number, however, the Heilborn hypothesis of the specific barriers being formed by only a pair or two of chromosomes is still valid. This is certainly so within the *Capillares* section, which, despite many specimens having been studied from each taxon, shows no variation in number within the taxon, while morphologically and geographically distinct taxa may show great or small differences in chromosome number.

On the basis of the above cytological results and the theoretical inferences the taxonomical subdivisions of the *Capillares* section may be evaluated. There is no doubt that the section itself is a distinct group lacking very evident relationships to other sections of the subgenus *Carex*. But its taxa have reached the species level in different ways and several units are still to be regarded only as intraspecific groups in process of differentiation.

From the point of view of sterility barriers, there is no doubt as to the specific barriers between the diploid, tetraploid, and hexaploid taxa of the *Capillares* section. The morphological variations and the distribution of several of the taxa are also strongly indicative of their taxonomic status. The diploids *C. Williamsii* and *C. Novograblenovii* were originally described from each side of the Beringian area, but although they certainly are very distinct from other species of the section, their morphological differences are nonexistent or so small that the present writers do not hesitate to regard them as a single taxon at the species level (152, 178).

At the exact tetraploid level are the taxa *C. Krausei* and *C. capillaris* var. *Porsildiana*. Though morphologically distinct and clearly distinguishable from

other elements usually included in *C. capillaris* s.str. (300), they seem to be geographical races of the same species only, and since they are regional rather than local, they are to be regarded as subspecies of *C. Krausei* (220). The typical race is restricted to parts of Alaska, while the ssp. *Porsildiana* is found from arctic Alaska in the west to northern Iceland in the east (cf. map in Löve and Löve (220)).

Cytologically, though not morphologically, close to *C. Krausei* are the hypertetraploids *C. Chamissonis* and *C. tenuiformis*. Both are so distinct that their specific status cannot be challenged, and their cytological differences must be taken as additional support for their distinctiveness. *C. Chamissonis* is a species of small variability and restricted distribution, whereas *C. tenuiformis* is a plant of considerable diversity and rather wide distribution in eastern Asia. On morphological grounds the present writers distinguish between the two subspecies, ssp. *neo-filipes* and ssp. *tenuiformis*, the latter of which also includes the two formae *puberula* and *paupera*. Only the typical taxon has been cytologically studied so far but there is no reason to suppose that the other taxa of the species are not characterized by the same chromosome number.

The exactly hexaploid taxa characterized by the chromosome number $2n = 54$ have been named as different species by several authors and as varieties only by others. The present writers are of the opinion that they all belong to the species *C. capillaris* L. The choice of the correct epithets for these taxa and some of the related species is by no means an easy task. Although the present writers regard the arctic–alpine plant to be the typical race of the species, they are not quite convinced that Linnaeus' type could not have been the tall-grown plant of the lowlands, here named as ssp. *chlorostachys*. The habitat of *C. capillaris* given by Linnaeus (204) is "pratis humidis", which is in contrast to the alpine low-grown type of tundras, summits of mountains, and exposed places on northern lowlands.

Drejer (80) in Flora Danica (Tab. 2374) depicted two races of *C. capillaris* from Greenland with the following text:

"Fig. 1 et 2: var. α tenuior, spicis subquaternis, terminali mascula, femineis subsexfloris, perigyniis griseofusca, angulis a albida. Drej. l.c.

Fig. 3: var. β major et robustior (subpedalis) spicis suboctonis, terminali androgyna (apice v. rarius basi feminea) femineis subdecemfloris, perigyniis minoribus pallidioribus longe stipitatis ovato-oblongis, caryopsis concolore grisea. Drej. l.c."

This l.c. refers to page 47 of the well-known "Revisio critica Caricum borealium" by Drejer (79).

It is worth while to note that although the two words *tenuior* and *major* are often used as names of varieties attributed to Drejer, no formal names are assigned by him to the two extremes. On the same page, there is also a *Carex filipendula* γ *concolor* (Drej.), which clearly shows his usual procedure when naming varieties. It seems as if Lange (191) in his "Conspectus Florae

Groenlandicae" is responsible for much of the difficulty by attributing the varietal names to Drejer, as seen by the following extract from the Conspectus:

> "*Carex capillaris* L. Drej. l.c. n. 44! Rink l.c. p. 117. Fl. Dan. tab. 2374! Teste Drejero l.c. in Groenlandia sub 2 formis occurrit:
>
> *α tenuior*, spica terminali mascula, foemineis c. 3, subsexfloris, perigyniis stipitatis, ellipticis, fuscis; caryopside variegata, lateribus griseo-fusca, angulis alba (Fl. Dan. l.c. Figs. 1–2).
>
> *β robustior*, 1′ longa, spica terminali androgyna, foemineis 6–7, subdecemfloris, perigyniis minoribus, longe stipitatis, ovato-oblongis, pallidioribus; caryopside concolore griseo (Fl. Dan. l.c. Fig. 3)."

The former variety is what the present writers regard as the typical race of the species *C. capillaris*. It is a small plant, 2–10 cm. high, with few-flowered spikes, and its distribution is arctic–alpine. The latter taxon was later wrongly named by Blytt (24) as var. *major* "Drejer" and identified with the race of the species met with in lower altitudes in damp, springy, or mossy places, often in forests. That latter type is here named as ssp. *chlorostachys*, since it is found to be identical with *C. chlorostachys* of Eurasia, *C. capillaris* var. *elongata* of North America, and some other taxa listed as synonyms below. It is not very common in Europe, although it seems to appear in Scandinavia (our forest type from Åhus, cf. the cytological chapter) and east of the Alps (cf. Muravyeva (249)), becoming increasingly frequent until it replaces the ssp. *capillaris* entirely in eastern Asia, together with the also tall-grown *C. Karoi*. It is very common across Canada and the northern United States and reaches considerable size especially in the central parts of the continent. Both Fernald (88, 89) and Porsild (292), among others, have understood very well its systematic value and its ecological and geographical significance. The same pattern of distribution as is observable for the two subspecies of *C. capillaris* (an arctic–alpine race contrasted with a subarctic–subalpine or lowland taxon) is very similar to that of *C. norvegica* Retz. and the closely related *C. media* R. Br. In both cases the taxonomical picture has also been quite confused, since *C. alpina* of old authors has long been misunderstood as has the present *C. capillaris*.

Three taxa were found to be characterized by the hyperhexaploid number $2n = 56$, while one taxon had as many as $2n = 58$ chromosomes. All these taxa have sometimes been regarded as only varieties of *C. capillaris*, but since they are not only cytologically but also morphologically and geographically distinct, they are certainly not identical with the typical species of the section. Two of the taxa with $2n = 56$ chromosomes (*C. Sedakowii* and *C. capillaris* ssp. *robustior*) are characterized by exactly the same karyotype, with the same number of large, medium, and small chromosomes, whereas *C. Ledebouriana* is similar in number but differs clearly in karyotype. Although all the hyperhexaploids most probably have developed from a common 54-chromosome stock, it is very unlikely that they have evolved from the same 56-chromosome taxon which later became extinct between Greenland and eastern Asia. Much more likely is the suggestion that the similarity of their karyotypes is

only apparent and that while both have originated by fragmentation of chromosomes from the same size class, different chromosomes have been involved and the fragmentation has occurred at very different times. *C. Sedakowii* is an old and well established species, while the Greenland taxon is of a more recent date and considerably less differentiated. In synonymy with *C. Sedakowii* the species *C. Chaffanjonii* Camus is tentatively placed, since it seems to be only a depauperate extreme of that taxon. According to Camus (47), it is closely related to *C. Sedakowii*, but differs because the female spikes are often reduced to a single one only, though with more flowers, the scales are more acuminate, and the stigmas longer. It was described from material collected in the lower valley of the Kerulen River in Mongolia.

The 56-chromosome Greenland plant, *C. capillaris* ssp. *robustior*, is a tall taxon with gynoecandrous spikes, short erect lateral spikes, and 2–3 mm. long perigynia, and, as pointed out by Böcher (29, 30), it is a continental and calciphilous plant. Böcher (29) showed it to be equally distinct from typical *C. capillaris*, as are *C. Krausei* and its ssp. *Porsildiana*, both of which were named by him as subspecies equal to his ssp. *robustior*. The cytological differences, however, support the view that this taxon is better regarded as a good species, although its more recent origin, as indicated by its small area of distribution, is probably why it does not yet differ from the other taxa in as many conspicuous characters as do the other species. It is undoubtedly identical with the var. β of Drejer (80). Böcher (29) named it *C. capillaris* ssp. *robustior* ("Drejer" ex Lange) Böcher, with the alternate: *C. capillaris* ssp. *major* ("Drejer") Böcher. The words "major et robustior" are only the beginning of a description by Drejer, certainly not meant to be formal epithets, although raised as such by Lange (191) for the Greenland plant, and by Blytt (24) for *C. capillaris* ssp. *chlorostachys* and incorrectly attributed to Drejer, as pointed out above. Since the use of the epithets *major* or *robustior* at the specific level is either prevented by a former homonym (*C. major* A. & Gr.) or doubtful because of other reasons, the present writers are of the opinion that in this special case the Recommendation 60A, 2 of the International Code (193) should not be followed and a new name should be created for the species. Therefore, the new name *Carex Boecheriana* is proposed for this Greenland endemic, in honor of Professor Tyge W. Böcher of Copenhagen, the distinguished specialist in all aspects of the Greenland flora and who most recently demonstrated the distinction of this taxon. Although a nice addition to the few sexual species among the thirty-odd Greenland endemics (31), the species should be looked for also in sheltered and continental regions of the Canadian Eastern Arctic, but the report of it from Iceland by Drejer (80) is probably a mistake.

The 58-chromosome taxon of the *Capillares* section is *C. Karoi* from eastern and central Asia. It is a tall-growing plant of continental regions, and although it has been mixed with *C. capillaris* ssp. *chlorostachys* or given different

specific or varietal names by several authors, it is distinct but rather variable. Only one variety from China seems to be worthy of retaining as a separate unit. This is the var. *parvirostris*, characterized by its short, ovate, pale, rather dull-colored, and subrugulose perigynium with a very short beak. It was originally described from the Chili province by Kükenthal (183) as a variety of *C. capillaris*, later reported from central Shansi by Kükenthal (184), and more recently named as species, *C. Licentii*, from the southern part of the Shansi province by Nelmes (255).

A key and a detailed review of the taxa of the *Capillares* section and their synonyms are given below.

Conspectus Caricum Sectionis Capillarium Ascherson & Graebner

A. Leaf-blades strongly canaliculate, 0.25–0.75 mm. wide; perigynia 2–3.5 mm. long.
  B. Spikelets 3–4, remote, the terminal one androgynous, the lateral ones loosely 3–7-flowered, drooping on long capillary pedicels; bracts tubulate-sheathing; perigynia twice as long as the scale, ellipsoid, 2–2.5 mm. long, attenuate into a short smooth beak; plant 10–50 cm. high. . . . . *C. Sedakowii* C. A. Meyer
  BB. Spikelets 3–6, the terminal one staminate, the 3–5 others pistillate, the upper two from the same sheath and approximate, the remaining ones more or less apart, erect and mostly on peduncles shorter than the spikes or about their length; lowest bract leaf-like, tubulate sheathing; perigynia twice as long as the scale, lanceolate, 2.5–3.5 mm. long, tapering into a smooth beak 1 mm. long; plant 5–15 cm. high. . . . . *C. Williamsii* Britton

AA. Leaf-blades flat, 1–4 mm. wide; perigynia 2.5–5.5 mm. long.
  C. Terminal spike 10–15 mm. long, mostly staminate, clavate-linear, pedunculate and overtopping the female ones.
    D. Lateral spikes loosely 5–10-flowered; Asiatic species.
      E. Lateral spikes 10–15 mm. long, few-flowered, the lowest bract leaf-like; scales oblong-ovate, roundish or acuminate at the tip, clearly hyalinous-margined, the keel often hispid; perigynia 2.5–3 mm. long, about the size of the scale, ovate in shape, the three angles well marked, nerveless, stipitate at base and attenuate into a cylindric obliquely-cut beak, yellowish, ciliate on the angles in the upper part; plant 20–25 cm. high. . . . . *C. Ledebouriana* C. A. Meyer
      EE. Lateral spikes 10–25 mm. long, few-flowered; stem more or less compressed, nodding at tip, fibrillose at base, the fibers brownish or even purplish; scales a little acuminate at tip; perigynia ovate-fusiform or lanceolate-fusiform, 3–3.5 mm. long, abruptly contracted into a long beak, glabrous or sometimes setulose; plant 15–40 cm. high. . . . . *C. tenuiformis* Lév. & Vaniot
        F. Perigynia ovate-fusiform, 3–4.5 mm. long, abruptly contracted into a beak shorter than the body. . . . . ssp. *tenuiformis*
          G. Perigynia glabrous and nerveless. . . . f. *tenuiformis*
          GG. Perigynia puberulent-setulose. . . . . f. *puberula* Akiyama
          GGG. Spikelets smaller. . . f. *paupera* (Kük.) L., L. & R.
        FF. Perigynia lanceolate-fusiform, 5–5.5 mm. long, the beak as long as the body. . . . . ssp. *neo-filipes* (Nakai) L., L. & R.
    DD. Lateral spikes densely 15–30 flowered; perigynia ovate-oblong, 2–2.5 mm. long, with a very short beak; scales about 2 mm. long, fuscous, with the margin hyaline and erose, articulate and soon deciduous; plant 35–60 cm. high. . . . . *C. Karoi* (Freyn) Freyn
      H. Perigynium ovate, about 2–2.5 mm. long, with a short and conical beak. . . . . var. *Karoi*
      HH. Perigynium barely 1.5 mm. long, ovate, pale, rather dull-colored, subrugulose, with a very short beak. . . . . var. *parvirostris* (Kük.) L., L. & R.

CC. Terminal spike linear, either staminate or gynoecandrous, not above the others.
I. Terminal spike usually gynoecandrous.
J. Leaves about the length of the culm; densely caespitose and fibrillose, the fibers often purplish; spikes 6–7, the terminal one with a few male flowers, the others entirely female, the basal one often ramose (bi- or tri-furcate), all on long peduncles, laxly-flowered and nodding; perigynia 2–2.5 mm. long, oblong-elliptic, attenuate at both ends, the three angles not prominent; scales obovate-obtuse, fulvous, with the mid-vein green and the margin hyaline, as long as the perigynium; plant 5–10 cm. high; Beringian species..... *C. Chamissonis* Meinshausen
JJ. Leaves shorter than the culms; spikes single; American species.
K. Tall plants, in fruit about 12–15 cm. high, then increasing to 24–30 cm. after the fruits have fallen off; lateral spikes 6–8 mm. long and 3 mm. wide, densely-flowered and erect; scales about 2 mm. long, orbicular, not promptly deciduous, castaneous with a large hyaline margin and erose at tip; perigynia 2.5–2.75 mm. long, ovoid, stipitate, contracted into a definite white-hyaline spinulose beak, the style-base indurated and exsert; endemic to Greenland..... *C. Boecheriana* L., L. & R.
KK. Smaller plants (at first 6–7 cm. high, growing up to 12 cm.) with perigynia 1.3–3.3 mm. long..... *C. Krausei* Böckeler
L. Perigynia 2.8–3.3 mm. long fusiform-ovoid, smooth; spikes 4–5, very short, about 8 mm. long, 2 mm. wide, long-peduncled and nodding; leaves usually well-developed..... ssp. *Krausei*
LL. Perigynia 1.3–1.8 mm. long, the lower part strongly distended over the sharply trigonous achene, the upper part then collapsing over the empty base and sometimes twisting the very short (0.3–0.5 mm. long) beak; spinules often present on the edges of the perigynia; lateral spikes 6–10, 6–8 mm. long and 2 mm. wide, erect..... ssp. *Porsildiana* (Polunin) L. & L.
II. Terminal spike usually staminate; perigynia oblong-ovate tapering to a long beak; circumpolar species..... *C. capillaris* L.
M. In dense tussocks; culms 2–20 cm. high; spikes 2–4, the lateral ones 6–8-flowered, 4–10 mm. long, 2–4 mm. broad; scales promptly deciduous; perigynia olivaceous, 2–3 mm. long, less than 1 mm. broad..... ssp. *capillaris*
MM. In looser tufts; culms 20–60 cm. high; spikes as much as 20-flowered, 7–17 mm. long; perigynia 2.5–4 mm. long..... ssp. *chlorostachys* (Steven) L., L. & R.

1. *Carex Sedakowii* C. A. Meyer, ex Meinshausen, Acta Horti Petrop. XVIII, 3: 360. 1901 (241).

Komarov (169): 376; Kükenthal (181): 588; (182): 156; Nakai (250): 323; (254): 131; Kreczetovicz (in 87): 129; (177): 427; Ohwi (268): 276; (269): 409; (270): 447; Kitagawa (162): 111; Raymond (300): 224; Akiyama (4): 139, tab. 125, fig. 1; Grubov (109): 88.

Carex capillaris var. *paludosa* F. Schmidt, Mém. Acad. St. Pétersb. Sér. 7, XII: 69, 1868 (319).

Carex Chaffanjonii E. G. Camus, in Lecomte, Not. System. II, 7 : 205, 1912 (47); Grubov (109): 82.

Western Siberia: Ob district, Altai. Eastern Siberia: Angara-Sajan, Dahuria, Lena-Kolyma. Far East: Zeya-Bureya, Udsk, Ussuri, Ochotsk, Northern Mongolia, Manshuria, Korea, and Kurile Islands.

2. *Carex Williamsii* Britton, in Britton & Rydberg, Bull. N.Y. Bot. Gard. 2: 159, 1901 (42).

Mackenzie (224): 296; (225): 348; Polunin (287): 124; Duman (81): 70; Hultén (149): 376; (150): 1726; Porsild (290): 19; (291): 78; (292): 115; Boivin (35): 202–203; Raymond (300): 222; Baldwin (14): 132; Dutilly, Lepage and Duman (83): 52; (84): 56; Ritchie (308): 291. – Non Kreczetovicz (178): 33 and map 25; cf. Hultén (152): 549.

Carex Novograblenovii Komarov, Bull. Jard. Bot. URSS 30: 199, 1932 (171); Kreczetovicz (177): 432.

Arctic Eastern Siberia, Anadyr (Osinovaya River), Tchukch (Arakamchatchene Island), Kamtchatka (heads of Pinatscheva and Nalotscheva Rivers), Ochotsk (Gichiga), Zeya-Bureya (Burei); Alaska and Yukon, the mouth of Mackenzie River, Keewatin District, west coast of Hudson Bay south to Cape Henrietta Maria, and northern Ungava.

3. *Carex Ledebouriana* C. A. Meyer, ex Treviranus, Bull. Soc. Nat. Moscou XXXVI, 1: 540, 1863 (384).

Kreczetovicz (in 87): 129; (177): 431; Grubov (109): 85.

Carex capillaris var. C. A. Meyer, in Ledebour, Fl. Alt. IV: 228, 1833 (194).
Carex capillaris β. Treviranus, in Ledebour, Fl. Ross. IV: 295, 1853 (195).
C. capillaris var. *Ledebourii* F. Schmidt, Mém. Acad. St. Pétersb. Sér. 7, XII: 69, 1868 (319). — Non Ohwi (270): 448.
Carex lenaeensis Kükenthal, Finska Vetensk. Soc. Förhandl. XLV, 8: 10, 1909 (180). — Non Kreczetovicz (177): 431.
Carex capillaris var. *Ledebouriana* Kükenthal, Pflanzenr. IV, 20: 591, 1909 (181); (182): 157. — Non Ohwi (270): 448.
Carex fraudulans et *Carex decipiens* Printz, Veg. Sib.-Mong. Front.: 160, fig. 81 et tab .IV, fig. 1 (295).

Western Siberia: Altai (Kuraisk). Eastern Siberia: Angara-Sajan, Dahuria, Lena-Kolyma (southern and eastern sectors). Far East: Anadyr, northern Mongolia.

4. *Carex tenuiformis* Léveillé & Vaniot, Bull. Acad. Intern. Géogr. Bot. 11: 104, 1902 (197).

Matsumura (237): 135; Kükenthal (181): 571; Miyabe and Kudo (246): 259; Akiyama (1): 177; (4): 140, pl. 126, fig. 1; Ohwi (268): 276; (270): 448; Raymond (300): 223; Nakai (254): 131.

Carex koreana Komarov, Acta Horti Petrop. 18: 446, 1901 (168); (169): 399; Kükenthal (181): 591; Nakai (250): 324; Koidzumi (166): 261; Akiyama (1): 179; Komarov & Klobukova-Alissova (173): 315, tab. 94; Kreczetovicz (in 87): 129; (177): 432. — Non *Carex coreana* Bailey (13): 8.
Carex seiskoensis Freyn, Österr. Bot. Zeitschr. LIII: 27, 1903 (95).
Carex Ichimurae Nakai, apud Matsumura, Shokubutsu-Mei, ed. rev. 81, 1916 (238).
Carex Komarovii Koidzumi, Bot. Mag. Tokyo, 33: 208, 1919 (167).
Carex capillaris Tatewaki, Journ. Jap. Bot. V: 38, 1928 (376); Miyabe and Kudo (246): 259, p.p. quoad pl. sachal. Non L.
Carex Inamii Ohwi, Acta Phytotax. & Geobot. XI, 4: 256, 1942 (271).
Carex tenuiformis var. *elatior* Ohwi (271), in syn.

4*a*. ssp. *tenuiformis*.

4*b*. f. *tenuiformis*.

Eastern Dahuria (Nerchinsk region), Ussuri, Manchuria, Korea, Sachalin, Kurile Islands, Yezo and Hondo.

4*c*. f. *puberula* Akiyama, J. Jap. Bot. XI: 495, 1935 (2); (4): 141, pl. 126, fig. 2; Ohwi (270): 449.

4*d*. f. ***paupera*** (Kükenthal) comb. nov.
C. koreana f. *paupera* Kükenthal, Pflanzenr. IV, 20: 571, 1909 (181).

Korea: Hallaisan. *Faurie 2295*.

4*e*. ssp. ***neo-filipes*** (Nakai) stat. nov.

Carex neo-filipes Nakai, Feddes Repert. XIII: 243, 1914 (251); (252): 517; (254): 131; Akiyama (4): 141, pl. 127.
Carex tenuiformis var. *neo-filipes* Ohwi ex Hatushima, Kiushiudaigaku-Enshiurin-Hokoku, V: 223, 1934 (124); (270): 449.

Korea: Mount Chiisan.

5. *Carex Karoi* (Freyn) Freyn, Österr. Bot. Zeitschr. XLVI: 132, 1896 (94).

Kreczetovicz (in 87): 129; (176): 89; (177): 430; Raymond (300): 224; Grubov (109): 85.
Carex capillaris ssp. *Karoi* Freyn, Österr. Bot. Zeitschr. XL: 303, 1890 (93).
Carex capillaris C. A. Meyer, in Ledebour, Fl. Alt. IV: 227, 1833 (194).
Carex delicata C. B. Clarke, Kew Bull. Add. Ser. VIII: 72, 1908 (66).
Carex capillaris f. *major* Kükenthal, Pflanzenr. IV, 20: 590, 1909 (181), p.p. non Drejer, excl. syn. var. *elongata* Olney et quoad specim. tibet. et sibir. sola; (182): 157; (183): 46; Tang (374): 144.
Carex capillaris ssp. *densiflora* Printz, Veg. Sib.-Mong. Front. 158, fig. 79 et 80, 1921 (295).
Carex displodens Printz, l.c. fig. 77, 1921 (295).
Carex Takenakai Nakai, J. Jap. Bot. XIX: 318, 1943 (253).

5*a*. var. *Karoi*

Asia: Irtish, Altai, Angara-Sajan, Dahuria, Lena-Kolyma, Zeya-Bureya, Dzungaro-Tarbagatai, Tian-Shan, Pamiro-Alaj, Kasachstania, Kuldja, northern Manchuria, Mongolia, western Tibet, China: Chili (Hopei).

5*b*. var. ***parvirostris*** (Kükenthal) comb. nov.

Carex capillaris var. *parvirostris* Kükenthal, Acta Horti Gotob. V: 46, 1929 (183).
Carex Licentii Nelmes, Kew. Bull. 1939: 185 (255).

China: Chili and Shansi.

6. *Carex Chamissonis* Meinshausen, Acta Horti Petrop. 18: 361, 1901 (241).

Kreczetovicz (177): 430; Hultén (149): 375; Raymond (300): 224.
Carex nana Chamisso, in Steudel, Synops. Cyper. 228, 1855 (338). Non Lamarck (190), nec Boott (36).
Carex capillaris var. *nana* Kükenthal, Pflanzenr. IV, 20: 591, 1909 (181), p.p. excl. spec. Krauseanum.
Carex capillaris Hultén, Fl. of Kamtchatka I: 204, 1927 (148), p.p. quoad spec. Redowskianum. Non L.

Asia: western shore of Okhotsk Sea at Gichiga and Ayan; Kamtchatka (?).

7. *Carex Krausei* Bœckeler, Bot. Jahrb. 7: 279, 1886 (33).

Bailey (12): 22; Holm (140): 6, 13; Hultén (149): 376.
Carex capillaris var. *Krausei* Kurtz, in Macoun, Cat. Can. Pl. IV: 163, 1888 (226); Raymond (300): 224.
Carex capillaris Kurtz, Bot. Jahrb. 19: 418, 1895 (186), p.p.; Mackenzie (224): 296, p.p. Non L.
Carex capillaris var. *nana* (Chamisso) Kükenthal, Pflanzenr. IV, 20: 591, 1909 (181), p.p. quoad spec. Krauseanum.
Carex capillaris var. *nana* f. *Krausei* (Boeck.) Kükenthal, l.c. (181).
Carex capillaris ssp. *Krausei* (Boeck.) Böcher, Medd. om Grönl. 147, 9: 52, 1952 (29); Böcher, Holmen and Jakobsen (31): 265.

7*a*. ssp. *Krausei*.

Alaska, cf. Hultén (149), and Löve and Löve (220, map on p. 175).

7*b*. ssp. *Porsildiana* (Polunin) Löve and Löve, Acta Horti Gotob. 20: 175, 1956 (220).

Carex capillaris var. *Porsildiana* Polunin, J. Linn. Soc. 52: 373, 1943 (288); Stefánsson and Steindórsson (337): 87.

Carex capillaris var. *Krausei* f. *Porsildiana* Raymond, Nat. Canad. 77: 59, 1950 (299); (300): 224.

Carex capillaris ssp. *Porsildiana* Böcher, Medd. om Grönl. 147, 9 : 52, 1952 (29); Böcher, Holmen and Jakobsen (31): 265.

Alaska, Hudson Bay reg., northern Ungava, Greenland, and Iceland; cf. map in Löve and Löve (220): 175.

8. ***Carex Boecheriana*** nom. et spec. nov.

Carex capillaris var. β, Drejer, Flora Danica, tab. 2374, fig. 3, and p. 9, 1849 (80).

Carex capillaris ssp. *robustior* ("Drejer" ex Lange) Böcher, Medd. om Grönl. 147, 9: 51–52, fig. 26, 1952 (29); Böcher, Holmen and Jakobsen (31): 264. Böcher, l.c., gives the alternate:

Carex capillaris ssp. *major* ("Drejer") Böcher, eodem loco, in nota, p. 52. Non *C. major* Ascherson and Graebner (8), nec var. *major* Blytt (24), nec f. *major* Kükenthal (181).

Endemic to Greenland.

9. *Carex capillaris* L., Spec. Plant. 977, 1753 (204), p.p.

Lightfoot (200): 557; Schkuhr (317): 97, tab. 0, fig. 56; Wahlenberg (396): 311; (397): 622; Hartman (112); (113); (114); (115); (116); (117); (118); (119); (120); (121); (122); (123); Hooker (143): 265; Schweinitz and Torrey (322): 350; Torrey (382): 416; Kunth (185): 472; Koch (164): 762; (165): 880; Boott (in 144): 227; (37): 182, p.p.; Drejer (79): 53; (80): 9 and tab. MMCCCLXXIV, p.p.; Babington (11): 345; Andersson (7): 32, tab. VII, fig. 77; Parlatore (283): 192; Steudel (338): 231; Carey (in 106): 527; Lilja (201): 661; Meinshausen (240): 413; (241): 362; Grönlund (107): 119; Bailey (12): 103, p.p.; Bentham and Hooker (20): 499; Macoun (226): 163; Hjelt (138): 305; Neuman and Ahlfvengren (257): 693; Stefánsson (335): 39; (336): 44; Stefánsson and Steindórsson (337): 87; Fernald (88): 509; (89): 356; Ostenfeld and Gelert (277): 90; Ascherson and Graebner (8): 153; Husnot (153): 40; Blytt and Dahl (25): 161; Simmons (326): 139; (327): 64; Rydberg (311): 74; (312): 134; (313): 184, p.p.; Robinson and Fernald (309): 246; Kükenthal (181): 590; Schinz and Keller (315): 99; Rouy (310): 452; Schinz and Thellung (316): 55; Vollmann (394): 115; Lindman (203): 154; Mackenzie (224): 295, p.p.; (225): Tab. 347; Vaage (388): 58; Sörensen (330): 109; (331): 43; Devold and Scholander (76): 116; Ostenfeld and Gröntved (278): 40; Gelting (100): 159; Kreczetovicz (177): 428; Victorin (391): 737, fig. 272, p.p.; Seidenfaden and Sörensen (324): 80; Böcher (26): 223; (29): 52; Polunin (286): 272; (287): 123; Mansfeld (235): 51; Nordhagen (262): 145; (263): fig. 462, p.p. dextra; Hermann (136): 49; Duman (81): 67, p.p.; Hylander (156): 27; (157): 35; Gröntved (108): 163; Hultén (149): 374, p. minim. p.; (151): 103, map 408; Lid (198): 154; (199): 176; Löve (208): 106; Fournier (92): 113; Nannfeldt (in 189): 291 and fig. 137; Almquist (5): 186; Dostál (78): 1902; Raymond (301): 416; (302): 5; Scoggan (323): 135, p.p.; Porsild (292): 113; (293): 93; Gleason (102): 344, p.p.; Clapham, Tutin, and Warburg (65): 1375; Binz and Thommen (22): 78; Szafer, Kulczynski and Pawlowski (345): 861; Weber (399): 74; Maevskij (227): 751; Böcher, Holmen, and Jakobsen (31): 263.

Carex pendula Genersich, Fl. Scep. Elench. no. 871, 1798 (101).

Carex plena Clairville, Man. 292, 1811 (64).

Carex capillaris var. α Drejer, Flora Danica tab. MMCCCLXXIV, figs. 1 and 2.

Carex capillaris f. *pygmaea* Huter, Exs. 1872 (nomen nudum) (cf. 8 and 181).

Carex capillaris α *tenuior* "Drejer", Lange, Medd. om Grönl. III: 148, 1880 (191).

Carex capillaris var. *minima* Beck, Fl. Nieder-Österr. 144, 1890 (19); Schinz and Thellung (316): 55.

Carex capillaris f. *alpestris* Norman, Fl. arct. Norv. Extr. 52, 1893 (264), nomen nudum; Blytt and Dahl (25): 162.

Carex capillaris f. *minima* Neuman and Ahlfvengren, Sv. Flora, 693, 1901 (257); Kükenthal (181): 590.

Carex fuscidula Kreczetovicz, Areal I: 32, 1952 (178).

9*a*. ssp. *capillaris*.

Arctic–alpine. Arctic Europe, arctic Asia, and arctic America. Mountains of Scotland and Scandinavia. Mountains of central Europe, south to the Pyrenees and Sierra Nevada. In America, south to mountains of New England in the east, and to Colorado and Utah in the west. Found also at sea-level, in exposed habitats, in eastern Quebec (Gaspé, Anticosti, etc.). Entirely missing on the mountains of Asia.

9*b*. ssp. ***chlorostachys*** (Steven) stat. nov.

Carex chlorostachys Steven, Mém. Soc. Nat. Moscou, IV: 68, 1813 (339); Marschall von Bieberstein (236): 615; Kreczetovicz (in 87): 129; (176): 89; (177): 428. Non Don (77).
Carex capillaris L. et plurim auct. p.p. quoad plantam altiorem subalpinam, sigillatim Wahlenberg, Hartman, Boott, Bailey, Meinshausen, Hjelt, Mackenzie, etc., loc. cit.; Treviranus in Ledebour (195): 295; Turczaninow (386): n. 1259; Trautvetter (383): 128; Boissier (34): 414; Christ (in 314): 177; Korshinsky (174): 410; Kurtz (187): 479; Britton and Brown (41): 320, p.p.; Neuman and Ahlfvengren (257): 693, p.p.; Komarov (169): 377; (170): 262; (172): 303; Meinshausen (241): 302, p.p.; Kükenthal (181): 590, p.p.; (182): 157, p.p.; Koidzumi (166): 143; Kudo (179): 84; Hultén (148): 204; (149): 374, p. maxim. p.; Miyabe and Kudo (246): 259; Rydberg (313): 184, p.p.; Akiyama (1): 179, fig 124; (3); (4): 140, pl. 125; Hiitonen (137): 166; Ohwi (270): 448; Raup (298): 134; Nakai (254): 129; Pojarkova (285): 132, tab. XLIV; Grubov (109): 83; Muravyeva (249): 118.
Carex saskatschewana Böckeler, Linnaea XLI: 159, 1877 (32); Bailey (13): 70.
Carex capillaris var. *elongata* Olney, ex Fernald, Proc. Amer. Acad. Sci. XXXVII: 509, 1902 (88); Robinson and Fernald (308): 246; Wooton and Stanley (406): 122; Porsild (292): 114.
Carex capillaris var. *major* "Drejer", Blytt, Norges Fl. 244, 1861 (24); Schinz and Thellung (316): 55.
Carex capillaris var. *major* "Blytt", Fernald, Gray's Man. 8th ed. 356, 1950 (89).
Carex capillaris C. *major* "Drejer", Ascherson and Graebner, Synops. Mitteleur. Fl. II, 2: 154, 1903 (8).
Carex capillaris f. *major* Blytt and Dahl, Haandb. i Norges Fl. 162, 1906 (25); Kükenthal (181): 590; (184): 114; Schinz and Thellung (316): 55; Tang (374): 144; Boivin (35): 203; Raymond (301): 417; non Drejer (79, 80).
Carex capillaris f. *saskatschewana* Kükenthal loc. cit. (181).
Carex lenaeensis Kreczetovicz in Fl. SSSR, III: 429. Non Kükenthal (180).
Carex capillaris var. *Ledebouriana* Ohwi (270); non C. A. Meyer (in 384).
Carex capillaris var. *Ledebourii* Ohwi (270); non F. Schmidt (319).
Carex selengensis N. Ivanova, Not. Syst. Herb. Inst. Bot. Kom. Acad. Sci. URSS, VII: 276, 1937 (158).

This subspecies grows in damp, springy, or mossy places at low altitude. It is not common in Europe but seems to appear in Scandinavia and east of the Alps, becoming increasingly frequent until in Asia it replaces entirely ssp. *capillaris*. It extends southward to Tian-Shan, Pamiro-Alaj, western Tibet, China, eastward to Manchuria, Korea, Yezo, Sachaline, and the Kurile Islands. It is found also at the tip of the Tchuck peninsula and across Bering Strait to Alaska, and is common across Canada south to the mountain, extending slopes of New Mexico.

Acknowledgment

Part of this work has been supported by generous grants from the National Research Council of Canada gratefully acknowledged by the first two authors.

References

1. Akiyama, S. Conspectus Caricum Japonicarum. A general view of Japanese *Carices* including those from Saghalien, the Kuriles, Hokkaido, Honshiu, Shikoku, Kiushu and Riukius, with special reference to utricles in female flowers. J. Fac. Sci. Hokkaido Univ. Ser. V, **2**, 1–266 (1932).
2. Akiyama, S. Notes on some Japanese *Carices*. IV. J. Japan. Bot. **11**, 494–499 (1935).
3. Akiyama, S. Species novae *Caricum* Japonicarum. II. J. Fac. Sci. Hokkaido Univ. Ser. V, **3**, 261–270 (1935).
4. Akiyama, S. Carices of the far eastern region of Asia. Publs. Fac. sci. Hokkaido Univ. (1955). pp. 1–257 and plates 1–248.
5. Almquist, E. Dalarnes flora. Stockholm. 1949.
6. Almquist, S. Om *Carex*-slägtets phylogenesis. Botan. Notiser, 1884.
7. Andersson, N. J. Cyperaceae Scandinaviae. Holmiae. 1849.
8. Ascherson, P. and Graebner, P. Synopsis der mitteleuropäischen Flora. Bd. 2.2. Leipzig. 1902–1904.

9. BABCOCK, E. B. The genus *Crepis*. Part one. The taxonomy, phylogeny, distribution, and evolution of *Crepis*. Univ. Calif. Publs. Botany, **21**, 1–197 (1947).
10. BABCOCK, E. B. The genus *Crepis*. Part two. Systematic treatment. Univ. Calif. Publs. Botany, **22**, 199–1030 (1947).
11. BABINGTON, C. C. Manual of British botany. London. 1843.
12. BAILEY, L. H. A preliminary synopsis of North American *Carices*, including those of Mexico, Central America and Greenland, with the American bibliography of the genus. Proc. Am. Acad. Arts Sci. **22**, (1886).
13. BAILEY, L. H. Studies on the types of various species of the genus *Carex*. Notes on *Carex* XI. Mem. Torrey Botan. Club, **1**, 1–85 (1889).
14. BALDWIN, W. K. W. Botanical investigations in the Reindeer – Nueltin lake area, Manitoba. Natl. Museum Can. Bull. **128**, 110–142 (1952).
15. BATTAGLIA, E. Assenza di centromero localizzato in *Heleocharis uniglumis* (Link) Schult. Caryologia, **6**, 319–332 (1954).
16. BATTAGLIA, E. A consideration of a new type of meiosis (mis-meiosis) in Juncaceae (*Luzula*) and Hemiptera. Bull. Torrey Botan. Club, **82**, 383–396 (1955).
17. BATTAGLIA, E. The concept of pseudopolyploidy. Caryologia, **8**, 214–220 (1956).
18. BATTAGLIA, E. and BOYES, J. W. Post-reductional meiosis: its mechanism and causes. Caryologia, **8**, 87–134 (1955).
19. BECK VON MANNAGETTA, G. R. Flora der Nieder-Österreich. Wien. 1890.
20. BENTHAM, G. and HOOKER, J. D. Handbook of the British flora. 5th ed. London. 1887.
21. BERGER, C. A. The cytology of *Luzula*. Am. J. Botany, **36**, 794 (1950).
22. BINZ, A. and THOMMEN, E. Flore de la Suisse. Lausanne. 1953.
23. BJÖRKMAN, S. O. *Zingeria biebersteiniana* (Claus) P. Smirn.—one more grass species with the chromosome number $2n = 8$. Svensk Botan. Tidskr. **50**, 513–515 (1956).
24. BLYTT, M. N. Norges flora. I. (Monocotyledoneae). Christiania. 1861.
25. BLYTT, A. and DAHL, O. Haandbog i Norges flora. Christiania. 1906.
26. BÖCHER, T. W. Biological distributional types in the flora of Greenland. A study on the flora and plant-geography of South Greenland and East Greenland between Cape Farewell and Scoresby Sund. Medd. om Grönland, **106** (2), 1–339 (1938).
27. BÖCHER, T. W. Zur Zytologie einiger arktischen und borealen Blütenpflanzen. Svensk Botan. Tidskr. **32**, 346–361 (1938).
28. BÖCHER, T. W. Contributions to the flora and plant geography of West Greenland. II. The *Carex capitata-*, the *Luzula multiflora-*, and the *Torularia humilis*-complexes. Medd. om Grönland, **147** (7), 1–39 (1950).
29. BÖCHER, T. W. Contributions to the flora and plant geography of West Greenland. III. Vascular plants collected or observed during the botanical expedition to West Greenland in 1946. Medd. om. Grönland, **147** (9), 1–85 (1952).
30. BÖCHER, T. W. Oceanic and continental vegetational complexes in southwest Greenland. Medd. om Grönland, **148** (1), 1–336 (1954).
31. BÖCHER, T. W., HOLMEN, K., and JAKOBSEN, K. Grønlands flora. Köbenhavn. 1957.
32. BÖCKELER, O. Die Cyperaceen des königlichen Herbariums zu Berlin. Linnaea, **41**, 145–356 (1877).
33. BÖCKELER, O. Neue Cyperaceen von Argentinien, Mexico, Alaska und dem Kilimandscharo. Botan. Jahrb. **7**, 273–280 (1886).
34. BOISSIER, E. Flora orientalis. V. Genevae et Basileae. 1884.
35. BOIVIN, B. Centurie de plantes canadiennes. II–IV. Naturaliste can. **75**, 202–227 (1948).
36. BOOTT, F. Illustrations of the genus *Carex*. I. London. 1858.
37. BOOTT, F. Illustrations of the genus *Carex*. IV. London. 1867.
38. BOSEMARK, N. O. Cytogenetics of accessory chromosomes in *Phleum phleoides*. Hereditas, **42**, 443–466 (1956).
39. BRIEGER, F. G. and KERR, W. E. Sôbre o comportamento mitotico e meiotico de cromosômas policentricos ou com ponto de insercâo difuso. Anales escuela Sup. Agr. Luis de Queiroz Mex. **6**, 179–192 (1949).
40. BRIQUET, J. Internationale Regeln der botanischen Nomenklatur. Dritte Ausgabe. Jena. 1935.
41. BRITTON, N. L. and BROWN, A. An illustrated flora of the northern United States and the British possessions. New York. 1896.
42. BRITTON, N. L. and RYDBERG, P. A. An enumeration of the flowering plants collected by R. S. Williams and by J. B. Tarleton. Bull. N.Y. Botan. Gardens, **2**, 149–187 (1901).
43. BROWN, S. W. Spurious secondary association and asymmetric spindles in a *Luzula*. Cytologia, **15**, 259–268 (1950).
44. BROWN, S. W. Mitosis and meiosis in *Luzula campestris* DC. Univ. Calif. Publs. **27**, 231–278 (1953).
45. CAMARA, A. Progressos no estudo do centromero. Broteria, **20**, 5–34 (1951).

46. CAMARA, A. Advances in the centromere's problem. Caryologia, **6** (Suppl. Vol.), 254–271 (1955).
47. CAMUS, E. G. *Carex* de l'Asie orientale. Lecomte, Not. Syst. **2**, 205–207 (1912).
48. CASTRO, D. DE. Lampbrush chromosomes in plants. Portugaliae Acta Biol. Sér. A, **2**, 259–261 (1948).
49. CASTRO, D. DE. Notes on two cytological problems of the genus *Luzula* DC. Genet. iberica, **2**, 201–209 (1950).
50. CASTRO, D. DE. Instabilidade cromòsomica em *Luzula purpurea*. Agron. Lusitana, **16**, 195–210 (1954).
51. CASTRO, D. DE, CAMARA, A., and MALHEIROS, N. X-rays in the centromere problem of *Luzula purpurea* Link. Genet. iberica, **1**, 49–54 (1949).
52. CASTRO, D. DE, CAMARA, A., and MALHEIROS, N. X-rays in the centromere problem of *Luzula purpurea* Link. Hereditas (Suppl. Vol.), 548–550 (1949).
53. CASTRO, D. DE and MELLO-SAMPAYO, T. Observações sobre *Luzula purpurea* Link. Heterocromatina. Asinapsis. Broteria, **20**, 87–100 (1951).
54. CASTRO, D. DE and MELLO-SAMPAYO, T. Poliploidia induzida pontese fragmentãçao em *Luzula purpurea*. Genet. iberica, **5**, 3–22 (1953).
55. CASTRO, D. DE, and NORONHA-WAGNER, M. Nota sobre a perpetuação de fragmentos cromosómicos em *Luzula purpurea*. Agron. Lusitana, **14**, 96–99 (1952).
56. CASTRO, D. DE, and NORONHA-WAGNER, M. Two X-rays induced translocations in *Luzula purpurea*. Genet. iberica, **6**, 3–18 (1954).
57. CASTRO, D. DE, and NORONHA-WAGNER, M. Nota sobre autopoliploidia induzida em *Luzula campestris* DC. Agron. Lusitana, **18**, 123–126 (1956).
58. CHAKRAVORTI, A. K. Theory of fragmentation of chromosomes and evolution of species. Sci. and Culture (Calcutta), **13**, 309–312 (1947).
59. CHAKRAVORTI, A. K. Multiplication of chromosome numbers in relation to speciation in Zingiberaceae. Sci. and Culture (Calcutta), **14**, 137–140 (1948).
60. CHAKRAVORTI, A. K. Cytogenetical studies in Musaceae. I. Karyotype analysis of *Musa* and fragmentation of chromosomes as an evolutionary process in the genus. Proc. 36th Indian Sci. Congr. 141 (1949).
61. CHAKRAVORTI, A. K. Cytogenetical studies in Musaceae. II. Meiosis in some diploid species of *Musa* with special reference to secondary association of bivalents. Proc. 36th Indian Sci. Congr. 141–142 (1949).
62. CHAKRAVORTI, A. K. Cytogenetical studies in Musaceae. IV. Karyotype analysis of the parthenogenetic bananas and the cytological basis of their variation. Proc. 36th Indian Sci. Congr. 142–143 (1949).
63. CHAKRAVORTI, A. K. Origin of cultivated bananas of south east Asia. Indian J. Genetics and Pl. Breed. **11**, 34–46 (1951).
64. CLAIRVILLE, J. P. DE. Manuel d'herborisation en Suisse et en Valois, etc. Winterthur. 1811.
65. CLAPHAM, A. R., TUTIN, T. G., and WARBURG, E. F. Flora of the British Isles. Cambridge. 1952.
66. CLARKE, C. B. New genera and species of Cyperaceae. Kew Bull. Roy. Botan. Gardens, **8**, 1–196 (1908).
67. CLAUSEN, J. Stages in the evolution of species. Ithaca. 1951.
68. CLAUSEN, J., KECK, D. D., and HIESEY, W. M. Experimental studies on the nature of species. I. Effect of varied environments on western North American plants. Carnegie Inst. Wash. Publ. **250**, 1–452 (1940).
69. COSSON, E. and GERMAIN, E. Atlas de la flore des environs de Paris ou illustrations de toutes les espèces des genres difficiles et de la plupart des plantes litigieuses de cette région, avec des notes descriptives et un texte explicatif en regard. Paris. 1845.
70. DANSEREAU, P. The varieties of evolutionary opportunity. Rev. can. biol. **11**, 305–388 (1952).
71. DANSEREAU, P. Le coincement, un processus ecologique. Acta Biotheoretica, **11**, 157–178 (1956).
72. DARLINGTON, C. D. Chromosome botany. London. 1956.
73. DAVIES, E. W. The cytogenetics of *Carex flava* and its allies. Watsonia, **3**, 129–137 (1955).
74. DAVIES, E. W. Some new chromosome numbers in the Cyperaceae. Watsonia, **3**, 242–243 (1956).
75. DAVIES, E. W. Cytology, evolution and origin of the aneuploid series in the genus *Carex*. Hereditas, **42**, 349–365 (1956).
76. DEVOLD, J. and SCHOLANDER, P. F. Flowering plants and ferns of southeast Greenland. Skrifter Svalbard Ishavet Nr. **56**, 1–209 (1933).
77. DON, D. Prodromus florae Nepalensis, sive enumeratio vegetabilium, quae in itinere per Nepaliam proprie dictam et regiones conterminas, ann. 1802–1803. Detexit atque legit D.D. Franciscus Hamilton. Londini. 1825.
78. DOSTÁL, J. Kvêtena ČSR. Praha. 1950.

79. DREJER, S. Revisio critica Caricum borealium in terris sub Danico jacentibus inventarum. H. Kröyer's Naturhist. Tidsskr. **3**, 423–480 (1841).
80. DREJER, S. Tabula MMCCCLXXIV in Flora Danica 14. Köbenhavn. 1849.
81. DUMAN, M. G. The genus *Carex* in eastern arctic Canada. Cath. Univ. Am. Biol. Ser. **36**, 1–84 (1941).
82. DURIETZ, G. E. The fundamental units of biological taxonomy. Svensk Botan. Tidskr. **24**, 333–428 (1930).
83. DUTILLY, A., LEPAGE, E., and DUMAN, M. Contribution a la flore du bassin de la baie d'Ungava. Contrib. Arct. Inst. Cath. Univ. Wash. No. **4F**, 1–104 (1953).
84. DUTILLY, A., LEPAGE, E., and DUMAN, M. Contribution a la flore du versant occidental de la baie James, Ontario. Contrib. Arct. Inst. Cath. Univ. Wash. No. **5F**, 1–144 (1954).
85. EHRENBERG, L. Kromosomtalen hos några kärlväxter. Botan. Notiser, 430–437 (1945).
86. EHRHART, F. Beiträge zur Naturkunde. IV. Hannover und Osnabrück. 1789.
87. FEDTSCHENKO, B. A., SUBACHEV, V. N., and ROSHEVITZ, R. J. Flora transbaicalica. Pars 2. Monocotyledonae. Moskva and Leningrad. 1931.
88. FERNALD, M. L. The variation of some boreal *Carices*. Proc. Am. Acad. Sci. **37**, 495–512 (1902).
89. FERNALD, M. L. Gray's Manual of Botany. 8th ed. New York. 1950.
90. FERNANDES, A. Sobre a cariologia de algunas plantas da Serra do Gerês. Agron. Lusitana, **12**, 551–600 (1950).
91. FLOVIK, K. The chromosome number of some species of *Carex* and *Eriophorum*. Nytt Mag. f. Natur. **83**, 77–78 (1943).
92. FOURNIER, P. Les quatre flores de la France. Deuxième tirage. Paris. 1946.
93. FREYN, J. Plantae Karoanae; Aufzählung der von Ferdinand Karo im Jahre 1888 im baikalischen Sibirien sowie in Dahurien gesammelten Pflanzen. Österr. Botan. Z. **40**, 303–308 (1890).
94. FREYN, J. Plantae Karoanae dahuricae. Österr. Botan. Z. **46**, 131–137 (1896).
95. FREYN, J. Plantae Karoanae amuricae et zeaënse. Österr. Botan. Z. **53**, 21–30 (1903).
96. FRIES, E. Summa vegetabilium Scandinaviae. Sect. I. Upsaliae. 1846.
97. GARDÉ, A. and MALHEIROS-GARDÉ, N. Comportamento nucleolar na meiose de *Luzula campestris* (L.) DC. Broteria, **22**, 115–130 (1953).
98. GEITLER, L. Über die Kernteilung von *Spirogyra*. Arch. Protistenk. **71**, 79–100 (1930).
99. GEITLER, L. Chromosomenbau. Berlin. 1938.
100. GELTING, P. Studies on the vascular plants of East Greenland between Franz Joseph Fjord and Dove Bay (Lat. 73° 15′–76° 20′ N.). Medd. om Grönland, **101** (2), 1–340 (1934).
101. GENERSICH, S. Florae Scepusiensis elenchus sive enumeratio plantarum in comitatu Hungariae Scepusiensi eumque percurrentibus montibus carpathicis sponte crescentium. Leutschoviae. 1798.
102. GLEASON, H. A. The new Britton and Brown: Illustrated flora of the northeastern United States and adjacent Canada. New York. 1952.
103. GODWARD, M. B. E. Compound chromosomes and polyploidy in *Spirogyra*. Heredity, **5**, 158 (1951).
104. GODWARD, M. B. E. The diffuse centromere or polycentric chromosomes in *Spirogyra*. Ann. Botany, N.S. **18**, 143–156 (1954).
105. GODWARD, M. B. E. Irradiation of *Spirogyra* chromosomes. Heredity, **8**, 293 (1954).
106. GRAY, A. Manual of the botany of the northern United States. 3rd ed. New York. 1859.
107. GRÖNLUND, C. Islands Flora, indeholdende en Beskrivelse af Blomsterplantene og de höjere blomsterlöse Planter, samt en Fortegnelse over de lavere Planter. Köbenhavn. 1881.
108. GRÖNTVED, J. The Pteridophyta and Spermatophyta of Iceland. The Botany of Iceland IV, **1**, 1–427 (1942).
109. GRUBOV, V. I. Konspekt flory Mongol'skoi Narodnuoi Respubliki. Trudy Mongol'skoi Komissi 51. Moskva and Leningrad. 1955.
110. HÅKANSSON, A. Meiosis and pollen mitosis in X-rayed and untreated spikelets of *Eleocharis palustris*. Hereditas, **40**, 325–345 (1954).
111. HARLING, G. Die Chromosomenzahlen einiger *Carex*-Arten. Botan. Notiser, 1945, 114–116 (1945).
112. HARTMAN, C. J. Handbok i Skandinaviens flora. Stockholm. 1820.
113. HARTMAN, C. J. Handbok i Skandinaviens flora. 2: a uppl. Stockholm. 1832.
114. HARTMAN, C. J. Handbok i Skandinaviens flora. 3: e uppl. Stockholm. 1838.
115. HARTMAN, C. J. Handbok i Skandinaviens flora. 4: e uppl. Stockholm. 1843.
116. HARTMAN, C. J. Handbok i Skandinaviens flora. 5: e uppl. Stockholm. 1849.
117. HARTMAN, C. J. Handbok i Skandinaviens flora. 6: e uppl. utgiven av C. Hartman. Stockholm. 1854.

118. HARTMAN, C. J. Handbok i Skandinaviens flora. 7:e uppl. utgiven av C. Hartman. Stockholm. 1858.
119. HARTMAN, C. J. Handbok i Skandinaviens flora. 8:e uppl. utgiven av C. Hartman. Stockholm. 1861.
120. HARTMAN, C. J. Handbok i Skandinaviens flora. 9:e uppl. utgiven av C. Hartman. Stockholm. 1864.
121. HARTMAN, C. J. Handbok i Skandinaviens flora. 10:e uppl. utgiven av C. Hartman. Stockholm. 1870.
122. HARTMAN, C. J. Handbok i Skandinaviens flora. 11:e uppl. utgiven av C. Hartman. Stockholm. 1879.
123. HARTMAN, C. J. Handbok i Skandinaviens flora. 12:e uppl. utgiven av T. O. B. N. Krok. Stockholm. 1889.
124. HATUSHIMA, S. Kiuschiudaigaku-Enshiurin-Hokoku. V. Tokyo. 1934.
125. HEDBERG, O. Cytological studies in East African mountain grasses. Hereditas, **38**, 256–266 (1952).
126. HEILBORN, O. Zur Embryologie und Zytologie einiger *Carex*-Arten. Svensk Botan. Tidskr. **12**, 212–220 (1918).
127. HEILBORN, O. Die Chromosomenzahlen der Gattung *Carex*. (Vorläufige Mitteilung). Svensk Botan. Tidskr. **16**, 271–274 (1922).
128. HEILBORN, O. Chromosome numbers and dimensions, species-formation and phylogeny in the genus *Carex*. Hereditas, **5**, 129–216 (1924).
129. HEILBORN, O. Genetic cytology and genetics in *Carex*. Bibliographia Genet. **1**, 459–461 (1925).
130. HEILBORN, O. Chromosome studies in Cyperaceae. Hereditas, **11**, 182–192 (1928).
131. HEILBORN, O. Aneuploidy and polyploidy in *Carex*. Svensk Botan. Tidskr. **26**, 137–146 (1932).
132. HEILBORN, O. On the origin and preservation of polyploidy. Hereditas, **19**, 233–241 (1934).
133. HEILBORN, O. The mechanism of so-called secondary association between chromosomes. Hereditas, **22**, 167–188 (1936).
134. HEILBORN, O. Notes on chromosome associations. Cytologia, Fujii Jub. Vol. (1937).
135. HEILBORN, O. Chromosome studies in Cyperaceae. III–IV. Hereditas, **25**, 224–240 (1939).
136. HERMANN, F. J. The genus *Carex* in Michigan. Am. Midland Naturalist, **25**, 1–72 (1941).
137. HIITONEN, I. Suomen kasvio. Helsinki. 1935.
138. HJELT, H. Conspectus florae Fennicae. III. Acta Soc. pro Fauna et Flora Fennicae, **5**, 259–562 (1895).
139. HOLM, T. Studies in the Cyperaceae. XX. "Greges Caricum". Am. J. Sci. Ser. 4. **16** (1903).
140. HOLM, T. The genus *Carex* in northwest America. Beih. Botan. Centr. **22**, 1–29 (1907).
141. HOLMEN, K. Cytological studies in the flora of Peary Land, North Greenland. Medd. om Grönland, **128** (5), 1–40 (1952).
142. HOLMEN, K. and MATHIESEN, H. *Luz ula Wahlenbergii* in Greenland. Botan. Tidsskr. **49**, 233–238 (1953).
143. HOOKER, W. J. Flora scotica, or a description of Scottish plants, arranged both according to the artificial and natural methods. London. 1821.
144. HOOKER, W. J. Flora Boreali-Americana, or the botany of the northern parts of the British America. II. London. 1839.
145. HORNE, A. S. Nuclear division in the Plasmodiophorales. Ann. Botany, **44**, 199–231 (1930).
146. HUGHES-SCHRADER, S. Cytology of coccids (Coccoidea–Homoptera). Advances in Genet. **2**, 12–203 (1948).
147. HUGHES-SCHRADER, S. and RIS, H. The diffuse spindle attachment of coccids verified by the mitotic behavior of induced chromosome fragments. J. Exptl. Zool. **87**, 429–451 (1941).
148. HULTÉN, E. Flora of Kamtchatka and the adjacent islands. I. Kgl. Svenska Vetenskapsakad. Handl. Ser. 3, No. **5** (1), 1–346 (1927).
149. HULTÉN, E. Flora of Alaska and Yukon. II. Acta Univ. Lundensis, N.F. **38**, 131–412 (1942).
150. HULTÉN, E. Flora of Alaska and Yukon. X. Acta Univ. Lundensis, N.F. **46**, 1485–1902 (1950).
151. HULTÉN, E. Atlas över växternas utbredning i Norden. Stockholm. 1950.
152. HULTÉN, E. Areal (Cartographical material for the history of flora and vegetation). Issued by the USSR Academy of Science under the editorship of A. A. Grossheim (†) and B. K. Schischkin. Part 1 under the editorship of A. I. Tolmatchew. Leningrad and Moscow 1952. 43 pp. 39 maps. Responsible editor: M. M. Iljin. Svensk Botan. Tidskr. **47**, 547–551 (1953).

153. HUSNOT, T. Cypéracées. Descriptions et figures des Cypéracées de France, Suisse et Belgique. Cahan. 1905–1906.
154. HUTCHINSON, J. The families of flowering plants. II. Monocotyledons. London. 1934.
155. HUXLEY, J. Evolution, the modern synthesis. London. 1942.
156. HYLANDER, N. Förteckning över Skandinaviens växter, utgiven av Lunds Botaniska Förening. I. Kärlväxter. Lund. 1941.
157. HYLANDER, N. Förteckning över Nordens växter, utgiven av Lunds Botaniska Förening. I. Kärlväxter. Lund. 1955.
158. IVANOVA, N. A. *Carex* sp. nov. et *Poa* sp. nov. Mongoliae septentrionalis nec non transbaicaliae meridionalis. Not. Syst. Herbar. Inst. Botan. Komarov, Acad. Sci. **7**, 277–279 (1937).
159. JUEL, H. O. Beiträge zur Kenntnis der Tetradenteilung. Jahrb. wiss. Botan. **35**, 626–659 (1900).
160. KIHARA, H. and ONO, T. Chromosomenzahlen und systematische Gruppierung der *Rumex*-Arten. Z. Zellforsch. u. mikroskop. Anat. **4**, 475–481 (1926).
161. KING, G. C. "Diffuse" centromere, and other cytological observations on two desmids. Nature, **171**, 181 (1953).
162. KITAGAWA, M. Lineamenta florae Manshuricae. Repts. Inst. Sci. Research, Manchoukuo 3, Appendix 1, 1–488 (1939).
163. KNABEN, G. Chromosome numbers of Scandinavian arctic-alpine plant species. I. Blyttia, **8**, 129–155 (1950).
164. KOCH, W. D. J. Synopsis florae germanicae et helveticae. Francofurti ad Moenum. 1837.
165. KOCH, W. D. J. Synopsis florae germanicae et helveticae. Ed. sec. II. Lipsiae. 1844.
166. KOIDZUMI, G. Contributiones ad floram Asiae orientalis. Botan. Mag. Tokyo, **31**, 31–41, 128–143, 253–262 (1917).
167. KOIDZUMI, G. Genetic and floristic phytogeography of the alpine flora of Japan. Botan. Mag. Tokyo, **33** (193)–(222) (1919).
168. KOMAROV, V. L. Species novae florae Asiae orientalis (Manschuriae et Koreae borealis). Acta Horti Petrop. **18**, 417–449 (1901).
169. KOMAROV, V. L. Flora Manshuriae. I. Acta Horti Petrop. **20**, 1–559 (1901).
170. KOMAROV, V. L. Flora peninsulae Kamtschatka. I. Leningrad. 1927.
171. KOMAROV, V. L. Species novae plantarum orientis extremi URSS. Bull. Jard. Botan. Acad. Sci. **30**, 189–223 (1932).
172. KOMAROV, V. L. Flora polüostrova Kamtcatki. Pt. I. Leningrad. 1951.
173. KOMAROV, V. L. and KLOBUKOVA-ALISSOVA, E. N. Opredelitel' rastenij Dal'nevostochnogo kraia. 2. Leningrad. 1932.
174. KORSHINSKY, S. I. Plantas amurenses in itinere anni 1891 collectas enumerat novasque species describit. Acta Horti Petrop. **12**, 287–431 (1892).
175. KOYAMA, T. An enumeration of Hayata's Indo-China collection of Cyperaceae: *Carex* and *Cyperus*. Naturaliste can. **82**, 194–216 (1955).
176. KRECZETOVICZ, V. I. Clavis dichotoma Caricum Asiae mediae. Acta Univ. Asiae Mediae, Ser. VIII-*b*, Bot. **20**, 74–89 (1935).
177. KRECZETOVICZ, V. *Carex*. Flora SSSR **III**, 11–464 (1935).
178. KRECZETOVICZ, V. I. Dizjunkcii arkto-al'pijskich osok v evraziatskoj arktike i pricziny ich vozniknovenija. Areal, **1**, 32–35 (1952).
179. KUDO, Y. Flora of the island of Paramushir. J. Coll. Agr. Hokkaido Univ. **11**, 23–183 (1922).
180. KÜKENTHAL, G. Cariceae Cajanderianae. Liste der von Herrn A. K. Cajander im Jahre 1901 im Lenagebiete gesammelten *Kobresia*- und *Carex*-Arten. Öfv. Finska Vetensk. Soc. Förhl. **45** (8), 1–12 (1902–1903).
181. KÜKENTHAL, G. Cyperaceae–Caricoideae. Pflanzenreich IV, **20**, 1–824 (1909).
182. KÜKENTHAL, G. Cyperaceae Sibiriae subfamilia Caricoideae Pax. J. Russ. Botan. 1910, 73–94; 1911, 29–199 (1910–1911).
183. KÜKENTHAL, G. Plantae sinenses a Dre H. Smith annis 1921–22 lectae. XXII. Acta Horti Gotoburgensis, **5**, 33–49 (1930).
184. KÜKENTHAL, G. Cyperaceen aus China, gesammelt von Dr. H. Smith auf seiner zweiten Reise 1924. Acta Horti Gotoburgensis, **5**, 107–114 (1930).
185. KUNTH, C. S. Enumeratio plantarum omnium hucusque cognitarum, secundum familias naturales disposita, adjectis characteribus, differentiis et synonymis. 2. Stuttgardiae et Tubingae. 1837.
186. KURTZ, F. Die Flora des Chilcatgebietes im südöstlichen Alaska, nach den Sammlungen der Gebrüder Krause. Botan. Jahrb. **19**, 323–431 (1894).
187. KURTZ, F. Flora der Tschuktschenhalbinsel. Botan. Jahrb. **19**, 431–493 (1894).
188. LA COUR, F. L. The *Luzula* system analysed by X-rays. Heredity, **6** (Suppl. Vol.), 77–81 (1953).
189. LAGERBERG, T. Vilda växter i Norden. I. Andra omarbetade och utökade upplagan. Stockholm. 1947.

190. LAMARCK, J. B. A. P. M. DE. Encyclopédie méthodique. Botanique. III. Paris. 1789.
191. LANGE, J. Conspectus florae Groenlandicae. Medd. om Grönland, **3**, 1–214 (1880).
192. LANGE, M. Species concept in the genus *Coprinus*. Dansk Botan. Arkiv, **14** (6), 1–164 (1952).
193. LANJOUW, J. *et al.* International code of botanical nomenclature. Utrecht. 1956.
194. LEDEBOUR, C. F. VON. Flora Altaica. IV. Stuttgartiae. 1833.
195. LEDEBOUR, C. F. VON. Flora Rossica. IV. Stuttgartiae. 1853.
196. LEVAN, A. and LÖVE, Á. Different chromosome numbers within the collective species *Carex polygama*. Hereditas, **28**, 495–496 (1942).
197. LÉVEILLÉ, H. and VANIOT, E. Les *Carex* du Japon. Bull. Acad. Intern. Géograph. Botan. **11**, 27–32, 103–112, 175–187 (1902).
198. LID, J. Norsk flora. Oslo. 1944.
199. LID, J. Norsk flora. 2. utg. Oslo. 1952.
200. LIGHTFOOT, J. Flora scotica. London. 1777.
201. LILJA, N. Skånes flora. Stockholm. 1870.
202. LIMA-DA-FARIA, A. Genetics, origin and evolution of kinetochores. Hereditas, **35**, 422–444 (1949).
203. LINDMAN, C. A. M. Svensk fanerogamflora. Stockholm. 1926.
204. LINNAEUS, C. Species plantarum. Holmiae. 1753.
205. LITARDIÈRE, R. DE. Sur deux Graminées (Avénées–Airopsidées) à nombre chromosomique de base ègal à 4: les *Airopsis tenella* (Cav.) Coss. et DR. et *Periballia laevis* (Brot.) Asch. et Graebn. Compt. rend. **227**, 1071–1072 (1948).
206. LITARDIÈRE, R. DE. Nombres chromosomiques dans le genre *Holcus* L. Compt. rend. **228**, 1786–1787 (1949).
207. LITVINOV, D. I. Florae Turkestanicae fragmenta. II. Trav. Museum Botan. Acad. Sci. St. Pétersburg, **7**, 71–101 (1910).
208. LÖVE, Á. Íslenzkar jurtir. Kaupmannahöfn. 1945.
209. LÖVE, Á. Taxonomical evaluation of polyploids. Caryologia, **3**, 263–284 (1951).
210. LÖVE, Á. The taxonomical evaluation of types with different chromosome numbers. Proc. VIIth Intern. Bot. Congr. Stockholm (1950), 283–284 (1953).
211. LÖVE, Á. Cytotaxonomical remarks on some American species of circumpolar taxa. Svensk Botan. Tidskr. **48**, 211–232 (1954).
212. LÖVE, Á. Cytotaxonomical evaluation of corresponding taxa. Vegetatio, **5-6**, 212–224 (1954).
213. LÖVE, Á. The foundations of cytotaxonomy. VIII^{e} Congr. Intern. Botan. Paris (1954), Rapp. et Comm. Sect. **9-10**, 59–66 (1954).
214. LÖVE, Á. and LÖVE, D. Chromosome numbers of Scandinavian plant species. Botan. Notiser, 1942, 19–59 (1942).
215. LÖVE, Á. and LÖVE, D. Cytotaxonomical studies on boreal plants. II. Some notes on the chromosome numbers of Juncaceae. Arkiv Botan. 31B, **1**, 1–6 (1944).
216. LÖVE, Á. and LÖVE, D. Cytotaxonomical studies on boreal plants. III. Some new chromosome numbers of Scandinavian plants. Arkiv Botan. 31A, **12**, 1–22 (1944).
217. LÖVE, Á. and LÖVE, D. Chromosome numbers of Northern plant species. Iceland Univ. Inst. Appl. Sci. Dept. Agr. Rept. B, **3**, 1–131 (1948).
218. LÖVE, Á. and LÖVE, D. Geobotanical significance of polyploidy. I. Polyploidy and latitude. Portugaliae Acta Biol. Sér. (A), R. B. Goldschmidt Vol., 273–352 (1949).
219. LÖVE, Á. and LÖVE, D. Cytotaxonomical studies on the northern bedstraw. Am. Midland Naturalist, **52**, 88–105 (1954).
220. LÖVE, Á. and LÖVE, D. Cytotaxonomical conspectus of the Icelandic flora. Acta Horti Gotoburgensis, **20**, 65–290.(1956).
221. LÖVE, Á. and SARKAR, N. Cytotaxonomy and sex determination of *Rumex paucifolius*. Can. J. Botany, **34**, 261–268 (1956).
222. LÖVE, Á. and SARKAR, P. Cytotechnology. (Mimeographed.) Winnipeg. (1956).
223. LÖVE, D. Cytogenetic studies on dioecious *Melandrium*. Botan. Notiser, 1944, 125–213 (1944).
224. MACKENZIE, K. K. Cyperaceae, tribe 1, Cariceae. North Am. Flora, **18**, 1–478 (1931–1935).
225. MACKENZIE, K. K. North American Cariceae. Vol. II. New York. 1940.
226. MACOUN, J. Catalogue of Canadian plants: Part IV, Endogens. Montreal. 1888.
227. MAEVSKIJ, P. F. Flora srednei polos' evropeijskoij chasti SSSR. Moskva and Leningrad. 1954.
228. MALHEIROS, N. and CASTRO, D. DE. Chromosome number and behaviour in *Luzula purpurea* Link. Nature, **160**, 159 (1947).

229. MALHEIROS, N., CASTRO, D. DE, and CAMARA, A. Chromosomas sem centromero localizado. O caso de *Luzula purpurea* Link. Agron. Lusitana, **9**, 51–74 (1947).
230. MALHEIROS, N. and GARDÉ, A. Contribuiçoes para o estudo citologico do genero *Luzula* Link. Agron. Lusitana, **9**, 75–79 (1947).
231. MALHEIROS-GARDÉ, N. Algunos efectos de la morfina en la mitosis de la *Luzula purpurea* Link. Genet. iberica, **2**, 29–38 (1950).
232. MALHEIROS-GARDÉ, N. and GARDÉ, A. Fragmentation as a possible evolutionary process in the genus *Luzula* DC. Genet. iberica, **2**, 257–262 (1950).
233. MALHEIROS-GARDÉ, N. and GARDÉ, A. Agmatoploidia no genero *Luzula* DC. Genet. iberica, **3**, 155–176 (1951).
234. MALHEIROS-GARDÉ, N. and GARDÉ, A. Chromosome number in *Luzula multiflora* Lej. Genet. iberica, **4**, 91–94 (1952).
235. MANSFELD, R. Verzeichnis der Farn- und Blütenpflanzen des Deutschen Reiches. Ber. Deut. Botan. Ges. **58***a*, 1–323 (1940).
236. MARSCHALL VON BIEBERSTEIN, F. A. Flora Taurico-Caucasica. III. (Supplementum). Charkoviae. 1819.
237. MATSUMURA, J. Index plantarum japonicarum sive enumeratio omnium ex insulis Kurile, Yezo, Nippon, Sikoku, Kiusiu, Liukiu et Formosa hucusque cognitarum. 2. Tokyo. 1905.
238. MATSUMURA, J. Shokubutsu mei-i. II. Tokyo. 1916.
239. MAYR, E. Systematics and the origin of species. New York. 1942.
240. MEINSHAUSEN, K. F. Flora Ingrica, oder Aufzählung und Beschreibung der Blüthenpflanzen und Gefäss-Cryptogamen des Gouvernements St. Petersburg. St. Petersburg. 1878.
241. MEINSHAUSEN, K. F. Die Cyperaceen der Flora Russlands, insbesondere nach den Herbarien der Akademie der Wissenschaften. Acta Horti Petrop. **18**, 221–415 (1901).
242. MELDERIS, A. New taxa of Afroalpine grasses. Svensk Botan. Tidskr. **50**, 535–547 (1956).
243. MELLO-SAMPAYO, T., CASTRO, D. DE, and MALHEIROS-GARDÉ, N. Observacões sobre a autotetraploidia induzida pela colquicina em *Luzula purpurea* Link. Agron. Lusitana **13**, 1–11 (1951).
244. MEYER, C. A. Cyperaceae novae descriptionibus et iconibus illustratae. Mém. Acad. Imp. Sci. St. Pétersburg, div. sav. **1**, 195–230 (1831).
245. MICHALSKA, A. Cytological investigations on *Luzula*. Acta Soc. Botan. Polon. **22**, 169–186 (1953).
246. MIYABE, K. and KUDÔ, Y. Flora of Hokkaido and Saghalien. II. J. Fac. Agri. Hokkaido Univ. **26**, 81–278 (1931).
247. MÜNTZING, A. The evolutionary significance of autopolyploidy. Hereditas, **21**, 263–378 (1936).
248. MÜNTZING, A. Cytogenetics of accessory chromosomes (B-chromosomes). Caryologia, Suppl. Vol. 282–301 (1954).
249. MURAVYEVA, O. A. Flora Leningradskoj oblasti. Leningrad. 1955.
250. NAKAI, T. 1911. Flora koreana. II. J. Coll. Sci. Imp. Univ. Tokyo, **31**, 1–573 (1911).
251. NAKAI, T. Plantae novae Coreanae et Japonicae. I. Feddes Repert. **13**, 243–250 (1914).
252. NAKAI, T. New plants found in Japan and Chosen I. Botan. Mag. Tokyo, **28**, 517–520 (1914).
253. NAKAI, T. Notulae ad plantas Asiae orientalis (XXVIII). J. Japan. Botany, **19**, 312–319 (1943).
254. NAKAI, T. A synoptical sketch of Korean flora. Bull. Natl. Sci. Museum Tokyo, **31**, 1–152 (1952).
255. NELMES, E. XX. Decades Kewenses. Plantarum novarum in Herbario Horti Regii conservatum. Decas CXXXIII. Kew Bull. Roy. Botan. Gardens, 177–187 (1939).
256. NELMES, E. The genus *Carex* in Indo-China, including Thailand and Lower Burma. Mém. Museum Natl. hist. natur. N.S. B, Bot. **IV**, 83–182 (1955).
257. NEUMAN, L. M. and AHLFVENGREN, F. Sveriges flora. Lund. 1901.
258. NORDENSKIÖLD, H. The somatic chromosomes of some *Luzula* species. Botan. Notiser 1949, 81–92 (1949).
259. NORDENSKIÖLD, H. Cyto-taxonomical studies in the genus *Luzula*. I. Somatic chromosomes and chromosome numbers. Hereditas, **37**, 325–355 (1951).
260. NORDENSKIÖLD, H. The effect of X-ray treatment in dormant seeds of *Luzula pallescens* and *L. capitata*. Kgl. Lantbruks-Högskol. Ann. **22**, 257–267 (1955).
261. NORDENSKIÖLD, H. Cyto-taxonomical studies in the genus *Luzula*. II. Hybridization experiments in the *campestris-multiflora* complex. Hereditas, **42**, 7–73 (1956).
262. NORDHAGEN, R. Norsk flora. Oslo. 1940.
263. NORDHAGEN, R. Norsk flora. Illustrasjonsbind, annet hefte. Oslo. 1948.

264. NORMAN, J. M. Florae arcticae Norvegiae species & formae nonnulae novae v. minus cognitae plantarum vascularium. Christiania Videnskab. Selsk. Forhandl. for 1893, Nr. **16**, 1–59 (1893).
265. NORONHA-WAGNER, M. Subsidio para o estudo citológico do género *Luzula* D.C. Genet. iberica, **1**, 59–67 (1949).
266. NORONHA-WAGNER, M. and CASTRO, D. DE. Um cromosoma supranumerário em *Luzula campestris*. Scientia Genet. **4**, 149–153 (1952).
267. NORONHA-WAGNER, M. and CASTRO, D. DE. Interpretação dum comportamento meiótico observado em *Luzula*. Scientia Genet. **4**, 154–161 (1952).
268. OHWI, J. Florula Shikotenensis. III. Acta Phytotax. et Geobotan. **1**, 263–287 (1933).
269. OHWI, J. A list of the species of *Carex* growing in the northern Kuriles. J. Japan. Botany, **11**, 404–410 (1935).
270. OHWI, J. Cyperaceae Japonicae. I. A synopsis of the Caricoideae of Japan, including the Kuriles, Sagahalin, Korea, and Formosa. Mem. Coll. Sci. Kyoto Univ. Ser. B, **11**, 229–530 (1936).
271. OHWI, J. Symbolae ad floram Asiae orientalis. 18. Acta Phytotaxon. et Geobotan. **11**, 249–271 (1942).
272. OKSALA, T. Zytologische Studien an Odonaten. I. Chromosomenverhältnisse bei der Gattung *Aeschna* mit besonderer Berücksichtigung der postreduktionellen Teilung der Bivalents. Ann. Acad. Sci. Fennicae, IV, Ser. A, **4**, 1–64 (1943).
273. OKSALA, T. The concept and mechanism of chromosome reduction. Hereditas, **34**, 104–112 (1948).
274. OKSALA, T. Timing relationships in mitosis and meiosis. Caryologia, Suppl. Vol., 272–281 (1954).
275. OKUNO, S. Chromosome numbers of *Carex*. Japan. J. Genetics, **15**, 332–333 (1939).
276. OKUNO, S. On the chromosome numbers in the genus *Carex*. Japan. J. Genetics, **16**, 164–170 (1940).
277. OSTENFELD, C. H. and GELERT, O. Flora Arctica. I. Copenhagen. 1902.
278. OSTENFELD, C. H. and GRÖNTVED, J. The flora of Iceland and the Faeroes. Copenhagen. 1934.
279. OSTENFELD, C. H. and PAULSEN, O. A list of plants from inner Asia collected by Dr. Sven Hedin, determined by various authors and compiled by ..... *In* Sven Hedin: Southern Tibet, **6**, Part III, Botany, 25–100 (1922).
280. ÖSTERGREN, G. *Luzula* and the mechanism of chromosome movements. Hereditas, **35**, 445–468 (1949).
281. ÖSTERGREN, G. and PRAKKEN, R. Behaviour on the spindle of the actively mobile chromosome ends of rye. Hereditas, **32**, 473–494 (1946).
282. PAMPANINI, R. La flora des Caracorum. Bologna. 1930.
283. PARLATORE, F. Flora Italiana. Vol. II. Firenze. 1852.
284. PIZA, S. DE TOLEDO. The present status of the question of the kinetochore. Genet. iberica, **2**, 193–199 (1950).
285. POJARKOVA, A. I. Flora Murmanskoij oblastij. II. Moskva-Leningrad. 1954.
286. POLUNIN, N. Arctic plants in the British Isles: Enumeration of the species and varieties. J. Botany, **77**, 270–274 (1939).
287. POLUNIN, N. Botany of the Canadian eastern Arctic. Part I. Pteridophyta and Spermatophyta. Natl. Museum Can. Bull. **92**, 1–408 (1940).
288. POLUNIN, N. Contributions to the flora and phytogeography of southwestern Greenland: an enumeration of the vascular plants, with critical notes. J. Linnean Soc., Botany, **52**, 349–406 (1943).
289. PÓLYA, L. Magyarországi növényfajok kromoszómaszámai. II. Ann. Biol. Univ. Debrecen, **1**, 46–56 (1950).
290. PORSILD, A. E. Materials for a flora on the continental Northwest Territories of Canada. Sargentia, **4**, 1–79 (1943).
291. PORSILD, A. E. Vascular plants of Nueltin Lake, N.W.T. Natl. Museum Can. Bull. **118**, 72–83 (1953).
292. PORSILD, A. E. Botany of southeastern Yukon. Natl. Museum Can. Bull. **121**, 1–400 (1951).
293. PORSILD, A. E. The vascular plants of the western Canadian Arctic Archipelago. Natl. Museum Can. Bull. **135**, 1–226 (1955).
294. PRAKKEN, R. and MÜNTZING, A. A meiotic peculiarity in rye, simulating a terminal centromere. Hereditas, **28**, 441–482 (1942).
295. PRINTZ, H. The vegetation of the Siberian–Mongolian frontiers (the Sayansk region). Kgl. Norske Videnskab. Selskabs. Forh. Contrib. ad Floram Asiae Interioris pertin. ed. H. Printz. III: 1–458 (1921).
296. RAJUS, J. Historia plantarum, species hactenus editas aliasque insuper multas noviter inventas atque descriptas complectens. In qua agitur primo de plantis in genere, earumque partibus, accentibus et differentiis, etc. Tomus primus. Londini. 1686.

297. RANDOLPH, L. F. and MITRA, J. Chromosome numbers of *Iris* species. Bull. Am. Iris Soc. **140**, 2–12 (1956).
298. RAUP, H. M. The botany of southwestern Mackenzie. Sargentia, **6**, 1–262 (1947).
299. RAYMOND, M. Quelques entités mineures nouvelles de la flore du Québec. I. Naturaliste can. **77**, 55–71 (1950).
300. RAYMOND, M. Histoire et distribution de *Carex Williamsii* Britton. Naturaliste can. **77**, 222–227 (1950).
301. RAYMOND, M. Les Cypéracées de l'île Anticosti: *Carex* et *Kobresia*; Can. J. Research, C, **28**, 406–444 (1950).
302. RAYMOND, M. Sedges as material for phytogeographical studies. Mém. Jard. Botan. Montréal, **20**, 1–23 (1951).
303. RAYMOND, M. Cyperacées d'Indo-Chine. I. Naturaliste can. **82**, 145–165 (1955).
304. REESE, G. Ergänzende Mitteilungen über die Chromosomenzahlen mitteleuropäischer Gefässpflanzen. II. Ber. Deut. Botan. Ges. **66**, 65–73 (1953).
305. RESENDE, F. Consequências cito-genéticas do "Kinetochoro difuso". Bol. Soc. Port. Ciência Natl. **19**, 252–260 (1953).
306. RHOADES, M. M. and KERR, W. E. A note on centromere organization. Proc. Natl. Acad. Sci. **35**, 129–132 (1949).
307. RHOADES, M. M. and VILKOMERSON, H. On the anaphase movement of the chromosomes. Proc. Natl. Acad. Sci. **28**, 433–436 (1942).
308. RITCHIE, J. C. The native plants of Churchill, Manitoba, Canada. Can. J. Botany, **34**, 269–320 (1956).
309. ROBINSON, B. L. and FERNALD, M. L. Gray's Manual of botany. 7th ed. New York. 1908.
310. ROUY, G. Flore de France. XIII. Paris. 1912.
311. RYDBERG, P. A. Flora of Colorado. Fort Collins. 1906.
312. RYDBERG, P. A. Flora of the Rocky Mountains and adjacent plains. New York. 1917.
313. RYDBERG, P. A. Flora of the prairies and plains of central North America. New York. 1932.
314. SCHEUTZ, N. J. Plantae vasculares Jeniseenses. Kgl. Svenska Vetenskapsakad. Handl. **22** (10), 1–210 (1888).
315. SCHINZ, H. and KELLER, R. Flora der Schweiz. Zürich. 1909.
316. SCHINZ, H. and THELLUNG. Flora der Schweiz. Zürich. 1914.
317. SCHKUHR, C. Beschreibung und Abbildung der theils bekannter, theils noch nicht beschriebenen Arten von Riedgräsern nach eigenen Beobachtungen und vergrösserter Darstellung der kleinsten Theile. Wittenberg. 1801.
318. SCHKUHR, C. Beschreibung und Abbildung der theils bekannten, theils noch nicht beschriebenen Arten von Riedgräsern nach eigenen Beobachtungen und vergrösserter Darstellung der kleinsten Theile. Nachtrag, oder die zweite Hälfte der Riedgräser. Wittenberg. 1806.
319. SCHMIDT, F. Reisen im Amurlande und auf der Insel Sachalin, im Auftrage der kaiserlich-russischen geographischen Gesellschaft. Botanischer Theil. Mém. Acad. Sci. St. Pétersburg, VII, **12**, 1–227 (1868).
320. SCHRADER, F. Notes on the mitotic behavior of long chromosomes. Cytologia, **6**, 422–430 (1935).
321. SCHRADER, F. Mitosis. 2nd ed. New York. 1953.
322. SCHWEINITZ, L. D. VON and TORREY, J. Monograph of the North American species of the genus *Carex*. Ann. Lyc. Natl. Hist. N.Y. **1**, 283–373 (1824).
323. SCOGGAN, H. J. Flora of Bic and the Gaspé peninsula, Quebec. Natl. Museum Can. Bull. **115**, 1–399 (1950).
324. SEIDENFADEN, G. and SÖRENSEN, T. The vascular plants of Northeast Greenland from 74° 30′ to 79° 00′ N. lat., and a summary of all species found in East Greenland. Medd. om Grönland, **101** (4), 1–215 (1937).
325. SHARMA, A. K. and BAL, A. K. A cytological investigation of some members of the family Cyperaceae. Fyton, **6**, 7–22 (1956).
326. SIMMONS, H. G. The vascular plants in the flora of Ellesmereland. Rept. Sec. Norw. Arctic Expedition in the "Fram" 1898–1902, No. **2**, 1–197 (1906).
327. SIMMONS, H. G. A survey of the phytogeography of the arctic American archipelago. Rept. Sec. Norw. Arctic Expedition in the "Fram" 1898–1902, No. **19**, 1–36 (1913).
328. SIMONET, M. Recherches cytologiques et génétiques chez les *Iris*. Bull. Biol. Paris, **66**, 256–444 (1932).
329. SKOTTSBERG, C. Chromosome numbers in Hawaiian flowering plants. Arkiv Botan. Andra serien, **3**, 63–70 (1955).
330. SÖRENSEN, T. The vascular plants of East Greenland from 70° 00′ to 73° 30′ N. lat. Medd. om Grönland, **101** (3), 1–177 (1933).
331. SÖRENSEN, T. The flora of Melville Bugt. Medd. om Grönland, **124** (5), 1–70 (1943).
332. SORSA, V. Outlines of meiosis in the moss genus *Sphagnum*. Hereditas, **41**, 250–258 (1955).

333. SORSA, V. The quadripolar spindle and the change of orientation of the chromosomes in meiosis of *Sphagnum*. Ann. Acad. Sci. Fennicae, Ser. A, IV, **33**, 1–64 (1956).
334. STEBBINS, G. L. Variations and evolution in plants. New York. 1950.
335. STEFÁNSSON, S. Flóra Íslands. Kaupmannahöfn. 1901.
336. STEFÁNSSON, S. Flóra Íslands. 2. útgáfa, aukin. Kaupmannahöfn. 1924.
337. STEFÁNSSON, S. and STEINDÓRSSON, S. Flóra Íslands. 3. útgáfa, aukin. Akureyri. 1948.
338. STEUDEL, E. G. Synopsis plantarum glumacearum. II. Synposis plantarum Cyperacearum. Stuttgartiae. 1855.
339. STEVEN, C. Stirpes rariores in itinere Caucasio a 1810 lectae. Mém. Soc. Natl. Moscou, **4**, 49–72 (1813).
340. STOUT, A. B. The individuality of the chromosomes and their serial arrangement in *Carex aquatilis*. Arch. Zellforsch. **9** (1913).
341. STRASBURGER, E. Typische und allotypische Kernteilung. Ergibnisse und Erörterungen. Jahrb. wiss. Botan. **42**, 1–71 (1905).
342. STRASBURGER, E. Chromosomenzahl. Flora, **100** (1910).
343. SUOMALAINEN, E. The kinetochore and the bivalent structure in the Lepidoptera. Hereditas, **39**, 88–96 (1953).
344. SUOMALAINEN, H. O. T. An improved method for the use of crystal violet and allied dyes as chromosome stains. Arch. Soc. Vanamo **7**, 33–36 (1952).
345. SZAFER, W., KULCZYNSKI, S., and PAWLOWSKI, B. Rosliny Polskie. Warszawa. 1953.
346. TAKHTAJAN, A. L. Quelques problèmes de la morphologie évolutive des Angiosperms. Essais Botan. Acad. Nauk. SSSR, **II**, 763–793 (1954).
347. TANAKA, N. Chromosome studies in Cyperaceae. I. Cytologia, Fujii Jub. Vol., 814–821 (1937).
348. TANAKA, N. Chromosomes of *Scirpus lacustris* L. Japan. J. Genetics, **13**, 254 (1937).
349. TANAKA, N. Chromosome studies in Cyperaceae. II. *Scirpus lacustris* L. Cytologia, **8**, 515–520 (1938).
350. TANAKA, N. Chromosome studies in Cyperaceae. III. The maturation divisions in *Scirpus lacustris* L., with special reference to the heteromorphic pairing. Cytologia, **9**, 533–556 (1939).
351. TANAKA, N. Chromosome studies in Cyperaceae. IV. Chromosome numbers of *Carex* species. Cytologia, **10**, 51–58 (1939).
352. TANAKA, N. Chromosome studies in Cyperaceae. V. Pollen development of *Carex grallatoria* Maxim. var. *heteroclita* Kükenth. ex Matsum. Japan. J. Genetics, **15**, 153–157 (1939).
353. TANAKA, N. Cytological studies in the genus *Carex*. A collective review. Japan. J. Genetics, **15**, 96–104 (1939).
354. TANAKA, N. Aneuploidy and polyploidy in Cyperaceae. Science (Tokyo), **9**, 130–132 (1939).
355. TANAKA, N. Chromosome studies in Cyperaceae. VII. Chromosome number and pollen development of *Fimbristylis*. Botan. Mag. Tokyo, **53**, 480–488 (1939).
356. TANAKA, N. Chromosome studies in Cyperaceae. VI. Pollen development and additional evidence for the compound chromosome in *Scirpus lacustris*. Cytologia, **10**, 348–362 (1940).
357. TANAKA, N. Chromosome studies in Cyperaceae. VIII. Meiosis in diploid and tetraploid forms of *Carex siderosticta* Hance. Cytologia, **11**, 282–310 (1940).
358. TANAKA, N. Chromosome studies in Cyperaceae. IX. Structural hybridity observed in meiosis of *Carex lanceolata* Boott. Botan. Mag. Tokyo, **54**, 378–388 (1940).
359. TANAKA, N. Chromosome studies in Cyperaceae. X. Aneuploid plants of *Carex multifolia* Ohwi. Botan. Mag. Tokyo, **54**, 438–446 (1940).
360. TANAKA, N. Chromosome studies in Cyperaceae. XI. Meiosis in *Carex duvaliana* Franch. et Sav. Japan. J. Botany, **11**, 213–219 (1941).
361. TANAKA, N. Chromosome studies in Cyperaceae. XII. Pollen development in five genera, with special reference to *Rhynchospora*. Botan. Mag. Tokyo, **55**, 55–65 (1941).
362. TANAKA, N. Chromosome studies in Cyperaceae. XIII. Aneuploid plants of *Carex podogyna* Franch. et Sav. with special reference to abnormal pollen. Botan. Mag. Tokyo, **55**, 181–186 (1941).
363. TANAKA, N. Intraspecific aneuploidy in the genus *Carex*. Botany and Zool. **9**, 122–124 (1941).
364. TANAKA, N. Chromosome studies in Cyperaceae. XIV. Aneuploid plants of *Carex senanensis* Ohwi, *C. canescens* L. and of *C. stipata* Mühlenb. Botany and Zool. **9**, 889–894 (1941).
365. TANAKA, N. Chromosome studies in Cyperaceae. XV. Aneuploid plants of *Carex macroglossa* Franch. et Sav. and *C. parciflora* Boott. Botan. Mag. Tokyo, **55**, 218–225 (1941).

366. TANAKA, N. Chromosome studies in Cyperaceae. XIX. Chromosome numbers of *Carex* (*Vignea*). Med. and Biol. **2**, 215–219 (1942).
367. TANAKA, N. Chromosome studies in Cyperaceae. XX. Chromosome numbers of *Carex* (*Vignea*). Med. and Biol. **2**, 220–224 (1942).
368. TANAKA, N. Chromosome studies in Cyperaceae. XXI–XXIII. Chromosome numbers of *Carex* (*Eucarex*). Med. and Biol. **2**, 289–292, 293–296, 297–300 (1942).
369. TANAKA, N. Chromosome studies in Cyperaceae. XIV–XVI. Chromosome numbers of *Carex* (*Eucarex*). Med. and Biol. **2**, 417–420, 421–424, 425–428 (1942).
370. TANAKA, N. Aneuploid plants of *Carex oxyandra* Kudo. Japan. J. Genetics, **19**, 129–130 (1943).
371. TANAKA, N. Structural hybridities in the genus *Carex*. Japan. J. Genetics, **21**, 58–59 (1946).
372. TANAKA, N. The problem of aneuploidy. (Chromosome studies in Cyperaceae, with special reference to the problem of aneuploidy). Biol. Contrib. in Japan, No. **4**, 1–327 (1948).
373. TANAKA, N. Chromosome studies in the genus *Carex*, with special reference to aneuploidy and polyploidy. Cytologia, **15**, 15–29 (1949).
374. TANG, T. Preliminary study on the Cyperaceae of Hopei. Bull. Fan Mem. Inst. Biol. **3**, 133–151 (1932).
375. TARNAVSCHI, I. T. Die Chromosomenzahlen der Anthophyten-Flora von Rumänien mit einem Ausblick auf die Polyploidie-Problem. Bull. Jard. Museum Botan. Univ. Cluj, **28**, 1–130 (1948).
376. TATEWAKI, M. Notes on the alpine plants from southern Saghalien. J. Japan. Botan. **5**, 37–40 (1928).
377. THOMAS, P. T. Centromere precocity in *Luzula*. Heredity, **4**, 137–138 (1950).
378. TISCHLER, G. On some problems of cytotaxonomy and cytoecology. J. Indian Botan. Soc. **16**, 165–169 (1937).
379. TISCHLER, G. Die Chromosomenzahlen der Gefässpflanzen Mitteleuropas. Haag. 1950.
380. TISCHLER, G. Allgemeine Pflanzenkaryologie. 2. Hälfte: Kernteilung und Kernverschmelzung. Handbuch der Pflanzenanatomie **II**, 1-1040 (1951).
381. TISCHLER, G. Allgemeine Pflanzenkaryologie: Angewandte Pflanzenkaryologie. Handbuch der Pflanzenanatomie **II**, Ergänzungsband, 1–848 (1953–1956).
382. TORREY, J. Monograph of North American Cyperaceae. Ann. Lyc. Natl. Hist. N.Y. **3**, 339–451 (1836).
383. TRAUTVETTER, E. R. VON. Plantae Sibiriae borealis ab A. Czekanowsky et F. Mueller annis 1874 et 1875 lectae. Acta Horti Petrop. **5**, 1–146 (1877).
384. TREVIRANUS, L. C. Ad caricographiam Rossiam A. B. Ledebourio evulgatam supplementum. Bull. Soc. Natl. Moscou, **36**, 533–544 (1863).
385. TUCKERMAN, E. Enumeratio methodica Caricum quarundam. Schenectadiae (1843).
386. TURCZANINOW, N. Flora baicalensi-dahurica seu descriptio plantarum in regionibus cis- et transbaicalensibus atque in Dahuria sponte nascentium. Mosquae. 1855.
387. TUTIN, T. G. *Milium scabrum* Merlet. Watsonia, **1**, 345–348 (1950).
388. VAAGE, J. Vascular plants from Eirik Raude's Land. (East Greenland 71° 30′–75° 40′ Lat. N.). Skrifter Svalbard Ishavet Nr. **48**, 1–87 (1932).
389. VAARAMA, A. Cytological observations on *Pleurozium Schreberi*, with special reference to centromere evolution. Ann. Bot. Soc. Vanamo, **28**, **1**, 1–59 (1954).
390. VALENTINE, D. H. The units of experimental taxonomy. Acta Biotheoretica, **9**, 75–88 (1949).
391. VICTORIN, FRÈRE MARIE. Flore laurentienne. Montréal. 1935.
392. VILKOMERSON, H. The unusual meiotic behavior of *Elymus Wiegandii*. Exptl. Cell. Research, **1**, 534–542 (1950).
393. VIRKKI, N. Mitose und Meiose. Naturw. Rundschau, **9**, 352–355 (1955).
394. VOLLMANN, F. Flora von Bayern. Stuttgart. 1914.
395. WAHL, H. A. Chromosome numbers and meiosis in the genus *Carex*. Am. J. Botany, **27**, 458–470 (1940).
396. WAHLENBERG, G. Flora Upsaliensis. Upsaliae. 1820.
397. WAHLENBERG, G. Flora suecica. Upsaliae. 1833.
398. WALTERS, M. S. Atypical chromosome movement in meiotic anaphase of *Bromus pitensis* × *B. marginatus*. Am. J. Botany, **36**, 619–625 (1952).
399. WEBER, W. A. Handbook of plants of the Colorado front range. Boulder. 1953.
400. WEISMANN, A. Das Keimplasma. Eine Theorie der Vererbung. Jena. 1892.
401. WHITE, M. J. D. The chromosome cycle of *Ascaris megalocephala*. Nature, **137**, 783 (1936).
402. WINGE, Ö. The chromosomes. Their numbers and general importance. C. R. Lab. Carlsberg, **13**, 131–275 (1917).
403. WINGE, Ö. Das Problem der Jordan-Rosen'schen *Erophila*-Kleinarten. Beitr. Biol. Pflanz. **14**, 313–334 (1926).

404. WINGE, Ö. Taxonomic and evolutionary studies in *Erophila* based on cytogenetic investigations. C. R. Lab. Carlsberg, Sér. Physiol. **23**, 41–74 (1940).
405. WINKLER, H. Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. Z. Botan. **8**, 417–531 (1916).
406. WOOTON, E. O. and STANLEY, P. C. Flora of New Mexico. Contrib. U.S. Natl. Herb. **19**, 1–794 (1915).
407. WULFF, H. D. Karyologische Untersuchungen an der Halophytenflora Schleswig-Holsteins. Jahrb. wiss. Bot. **84**, 812–840 (1937).
408. WULFF, H. D. Chromosomenstudien an der schleswig-holsteinischen Angiospermen-Flora. I. Ber. Deut. Botan. Ges. **55**, 262–269 (1937).
409. WULFF, H. D. Chromosomenstudien an der schleswig-holsteinischen Angiospermen-Flora. III. Ber. Deut. Botan. Ges. **57**, 84–91 (1939).
410. WULFF, H. D. Chromosomenstudien an der schleswig-holsteinischen Angiospermen-Flora. IV. Ber. Deut. Botan. Ges. **57**, 424–431 (1939).
411. WULFF, H. D. Die Pollenentwicklung der Juncaceen nebst einer Auswertung der embryologischen Befunde hinsichtlich einer Verwandtschaft zwischen den Juncaceen und Cyperaceen. Jahrb. wiss. Botan. **87**, 533–556 (1939).
412. ZOHARY, D. Secondary centric activity in *Lilium formosanum*. Am. Naturalist, **89**, 50–52 (1955).

PROPERTIES OF A STRAIN OF CUCUMBER-MOSAIC VIRUS ISOLATED FROM PRUNUS HOSTS[1]

R. S. WILLISON[2] AND M. WEINTRAUB[2]

Abstract

Some of the stone-fruit viruses that are transmissible only to cucumber and other cucurbits have occasionally been accompanied by a second virus that can be isolated by transfer to tobacco. This virus, herein called CMVP, appears to be latent in *Prunus* hosts, but induces symptoms in bean, cowpea, cucumber, *Datura stramonium*, *Nicotiana glutinosa*, petunia, tobacco, spinach, sugar beet, Swiss chard, and zinnia. CMVP has a small thermal coefficient, a thermal inactivation point between 65° and 70° C., and a dilution end point between 10^{-3} and 10^{-4}. It remains infective in expressed sap up to 96 hours at room temperature and for more than 6 days under refrigeration. It can be transmitted between cucumber and tobacco by *Aphis gossypii* and *Myzus persicae*, in which it is nonpersistent. Spherical "virus particles" associated with CMVP are about 35 mμ in diameter. Tobacco plants infected with this virus are partially protected against cucumber mosaic virus but not against tobacco ring spot virus.

In symptom expression and in some of its properties, CMVP resembles both alfalfa mosaic virus and cucumber mosaic virus. Its particle size and immunological reaction suggest that it is an atypical strain of the latter. It is considered not to be implicated in the etiology of cherry yellows and related stone-fruit viroses.

Introduction

Recently a number of virus entities usually associated with diseases of herbaceous hosts have been reported as isolated from or transmitted to *Prunus* spp. and other woody plants. In 1952, Varney and Moore (13, 14), using as a source of inoculum immature leaves of American elm with mosaic symptoms, mechanically transmitted to tobacco a virus that they considered to be a strain of tomato ring spot. In the same year, Wilkinson (18) infected seedlings of a number of species of woody plants, including *Prunus avium*, with tobacco ring spot virus. *P. avium* plants reacted to infection with mosaic symptoms and stunting, but later became symptomless. The virus was recovered in tobacco after the first year of dormancy. Most of the other hosts used in the experiment continued to show symptoms after they had become systemically infected. In 1953, McWhorter (6) isolated from cherry petals a virus causing local lesions in primary leaves of cowpea (*Vigna sinensis*). Milbrath (7) also obtained a virus that induced necrotic local lesions on cowpea and small chlorotic local lesions with white centers on cucumber cotyledons when he used inoculum from petals of Montmorency cherry trees suspected of harboring latent virus. Leaf inoculum from the same trees, on the other hand, yielded the typical reaction of cucumber to the cherry-yellows–necrotic-ring-spot virus complex. Wilkinson (19) in 1953 found a strain of cucumber mosaic virus in naturally infected *Berberis thunbergii*. This strain induced etch-type ring spots on inoculated leaves of tobacco and a mild systemic mottling. Known strains of cucumber mosaic virus protected tobacco against subsequent

[1]Manuscript received May 25, 1957.
Contribution No. 1612 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2]Plant Pathology Laboratory, St. Catharines, Ontario.

infection by the barberry strain whereas tobacco plants infected with the barberry strain were not protected against a known strain of cucumber mosaic virus although symptom expression of the latter was delayed and reduced. In 1954, Varney and Moore (15) reported transmission of a virus from leaves of sour cherry, mahaleb cherry, peach, and Italian prune to tobacco and zinnia. Symptoms on tobacco consisted of concentric rings and line patterns; they were sometimes followed by true recovery. On zinnia, leaf symptoms ranged from faint temporary mottle mosaic to striking yellow and white mottling. This virus was not identified.

In the summer of 1950 at St. Catharines, 10 virus entities were isolated on cucumber from leaves of young peach or cherry trees bud-inoculated with stone-fruit viroses of the cherry-yellows–necrotic-ring-spot group from different sources. Later these isolates were inoculated into a series of herbaceous hosts. Seven of them produced definite symptoms only on cucumber, which reacted as expected. Three, however, induced symptoms like those of cucumber mosaic in cucumber, slight systemic vein clearing in tobacco and *Nicotiana glutinosa*, mild systemic mottle and vein clearing in petunia, and primary ring and spot lesions in *Datura stramonium*. A similar virus was also isolated later from a peach tree infected with cherry yellows, when the juice of hard-ripe fruit was used as inoculum. Leaf inoculum from the same tree, however, induced the typical cherry yellows reaction on cucumber. The atypical isolates were at that time considered to be contaminated with cucumber mosaic virus and were discarded and forgotten. In the light of later experience, however, it is probable that a strain of cucumber mosaic virus had actually been present as a latent infection in the *Prunus* hosts. The present paper deals with the isolation and properties of a similar entity, referred to as CMVP, strains of which were occasionally found in association with other stone-fruit virus infections. No symptoms that could be attributed to CMVP were observed on the *Prunus* hosts.

Materials

Sources of CMVP were two sets of four bud-inoculated peach trees, one set with green ring mottle virus strain G.1 from sour cherry and the other with tatter leaf virus strain T.2 from sweet cherry. Unless otherwise stated, *in vitro* experiments were conducted with cucumber plants of the variety Windermoor Wonder. The tobacco used throughout was the Burley variety Harrow Velvet. Other herbaceous hosts inoculated in host range studies are listed in the section dealing with symptomatology. Methods used in various phases of the investigation are described under the appropriate headings.

Isolation

In August 1951, seven different virus entities, prune dwarf strains D.2 and D.3, tatter leaf strain T.2, necrotic ring spot strains N.3 and N.6, and green ring mottle strains G.1 and G.2 were transferred mechanically from peach sources to cucumber. All induced in cucumber the type and range of symptoms recognized as those of the cherry-yellows–necrotic-ring-spot group

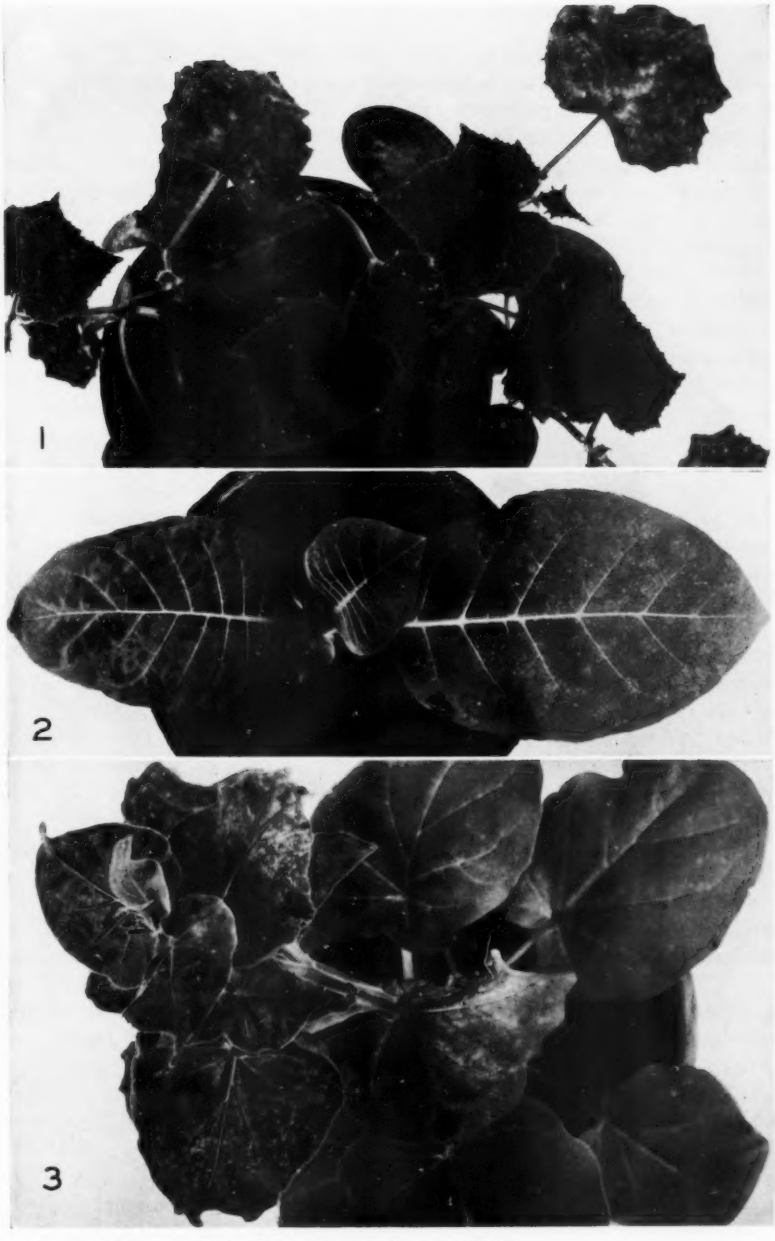


FIG. 1. Symptoms induced in cucumber by strain G.1B.
FIG. 2. Primary necrotic flecking and etching, and systemic chlorotic ring and vein-banding patterns in tobacco infected with strain G.1B.
FIG. 3. Chlorotic and necrotic flecking induced in *N. glutinosa* by strain T.2B.

of viruses (3, 5, 20). These isolates and others of longer standing were used in the *in vitro* experiments already reported (16, 21, 22). All except isolates T.2 and G.1 retained their identity and characteristics as long as they were maintained in cucumber and, in spite of numerous attempts, did not infect tobacco. Isolate T.2, however, with successive transfers to cucumber became milder in symptom expression and more resistant to aging *in vitro* and on the sixth transfer was transmitted to tobacco. Isolate G.1 also changed in much the same way as T.2 but more slowly. The changing identity of the two isolates suggested that each comprised at least two components, of which one, CMVP, was at first in relatively low concentration but in time superseded the other component. After transfer through tobacco, the two isolates, which for convenient reference may be designated T.2B and G.1B, behaved like strains of the same virus, although G.1B was, under similar conditions, somewhat more virulent than T.2B as well as less subject to attenuation after repeated subculturing in cucumber.

CMVP strains G.1B and T.2B have each been isolated from peach on four occasions, each time in conjunction with the stone-fruit virus entity. However, green ring mottle virus strain G.1 and tatter leaf virus strain T.2 have also been transmitted from peach to cucumber on 12 and 10 other occasions respectively when CMVP was not recovered, even after a number of attempts to infect tobacco with each of the isolates. This failure to isolate CMVP suggests either that its concentration was normally too low for it to compete successfully with the stone-fruit virus or that it was not uniformly systemic in affected trees. However, each time CMVP was isolated in association with the tatter leaf virus strain its concentration tended to increase in serial transfers through cucumber until it eventually supplanted the tatter leaf virus. On the other hand, the green ring mottle virus appeared to be better able to compete with CMVP. In three of the four cases in which the two viruses were isolated together, CMVP was displaced by the green ring mottle virus after 7 to 10 serial transfers through cucumber. For example, in one case the combination was inoculated to both cucumber and tobacco, after three, four, five, six, and seven successive transfers through cucumber. CMVP became established in and transmissible from tobacco in the third and fifth series, and gave dubious symptoms on tobacco in the fourth and sixth series and none in the seventh.

Inoculation to tobacco indicated the possibility that CMVP may also have been present in some isolations of cherry yellows virus strain Y.2, green ring mottle virus strain G.2, necrotic ring spot virus strain N.1, prune dwarf virus strain D.1, and tatter leaf virus strains T.1, T.3, and T.5. If so, it was at too low a concentration to become systemically established in tobacco.

Symptomatology

Both G.1B and T.2B, when inoculated to a number of species of herbaceous plants, induced similar symptoms on the whole, although some minor differences were occasionally noted. T.2B was usually slightly milder than G.1B in some but not all hosts. Symptoms are described in Table I.

TABLE I

SYMPTOMS INDUCED BY CMVP STRAINS G.1B AND T.2B ON DIFFERENT HOSTS

Host	Symptoms
Bean, *Phaseolus vulgaris*	*1. Yellowing and necrotic flecking; or none 2. Slight vein-banding or none
Cowpea, *Vigna sinensis*	1. No definite primary symptoms 2. Mottling and down-curling; infection usually doubtful
Cucumber, *Cucumis sativa*	1. Local lesions, chlorotic spots and rings, sometimes vein-banding 2. Rugose leaves, mosaic and spot patterns, slight dwarfing. Plants sometimes tend to recover (Fig. 1)
Datura stramonium	1. Pale to chlorotic spotting, transient green rings on chlorotic background 2. Inconspicuous mottling or none. Datura sometimes escaped infection
Nicotiana glutinosa	1. Yellow spots and areas, variable in intensity 2. Wrinkling and distortion of leaves and faint vein-banding to chlorotic and necrotic flecking. Plants tended to recover. Symptoms varied with conditions (Fig. 3)
Petunia hybrida	1. Inconspicuous mottling 2. Mild vein-clearing
Tobacco, *Nicotiana tabacum*	1. Chlorotic local lesions or necrotic flecking and etching often in irregular ring and line patterns. Variable with conditions 2. Mild vein-banding to necrotic flecking and etching or chlorotic ring and oak-leaf patterns, frequently zonate (G.1B). Variable in severity. Plants usually recovered in about two weeks after inoculation (Fig. 2)
Tomato, *Lycopersicum esculentum*	1. None observed 2. Inconspicuous mottling, or none. Tomato appeared to escape infection easily. The virus was recovered from tomato in only one of three trials
Snapdragon, *Antirrhinum majus*	1. Apparently not infected; if so, symptomless
Spinach, *Spinacia oleracea*	1. Yellow spots; general yellowing, sometimes with green rings (T.1B): rubbed leaves became necrotic from tip downward and died 2. Chlorotic and necrotic spotting, tip necrosis; young leaves small and narrow, plants stunted and sometimes killed
Sugar beet, *Beta vulgaris*	1. White to gray flecking 2. None observed; plants may have been somewhat stunted
Swiss chard, *Beta vulgaris cicla*	1. Pale to chlorotic flecking 2. None observed. Slight stunting?
Zinnia elegans	1. Inconspicuous or none 2. Slight mottling, vein-clearing, downward curling of leaves; or none Zinnia sometimes was a symptomless carrier and the virus was recovered from inoculated plants whether they showed symptoms or not

*1 = Primary symptoms. 2 = Systemic symptoms.

Properties

In the experiments reported below the infectivity of each preparation was tested on 5 to 10 cucumber plants by inoculating one cotyledon of each when the first true leaf was unfolding. Quantitative assays were scored by means of a numerical infection rating (21) based on the average severity of the disease induced in the group of plants inoculated with a given extract or aliquot thereof. An infection rating of 3 indicates that all plants in the group showed characteristic systemic symptoms. When no plants were infected the rating was 0. Intermediate values were obtained when some of the assay plants either failed to become infected or were less severely affected than usual.

Thermal inactivation of each strain was determined as described in a previous paper (16). Crude juice was diluted to 10^{-1} with distilled water and divided into aliquots which were exposed for 10 minutes to temperatures between 65° and 35° C. at five-degree intervals. The aliquots were kept in an ice-water bath before and after treatment until assay inoculations could be made. Tests with expressed cucumber sap were repeated seven times for strain G.1B and eight times for strain T.2B. Conditions for the growth of source plants varied considerably from test to test and, by affecting either virus concentration or the virus-inhibitor balance, or both, had a profound effect on the thermal inactivation point. For example when source plants were grown in the greenhouse under unfavorable winter conditions or were held at temperatures at or above 85° C. the inactivation point varied from 45° C. to about 55° C., whereas when the tests were made in spring or fall under a more favorable combination of conditions the inactivation point was approximately 65° C. The combined results of these tests (Table II) indicate not only a remarkable agreement in the response of the two isolates, but also progressive losses of infectivity after 10-minute exposures to temperatures above 40° C. Thus the two strains evidently have a small thermal coefficient (2), which, for lack of a good local lesion indicator, was not always detectable in individual tests (16). In an experiment carried out in May, 1952, with crude tobacco sap assayed on tobacco the inactivation point fell between 65° and 70° C. Appreciable losses in infectivity, indicated by moderation in severity and by delay in the appearance of systemic symptoms, occurred at temperatures at and above 55° C.

Resistance to aging and dilution end points varied with conditions in much the same way as did thermal inactivation. Under favorable conditions, crude cucumber juice diluted to 10^{-1} with water remained infective for periods up to 96 hours at room temperature and for more than six days when refrigerated.

TABLE II

AVERAGE INFECTION RATING OF ISOLATES G.1B AND T.2B IN CRUDE CUCUMBER LEAF EXTRACTS DILUTED TO 10^{-1} AND EXPOSED FOR 10 MINUTES TO VARIOUS TEMPERATURES

Isolate	Control	35° C.	40° C.	45° C.	50° C.	55° C.	60° C.	65° C.
G.1B	2.9	2.9	2.8	2.3	1.8	0.8	0.3	0.04
T.2B	3.0	2.9	2.8	2.5	2.0	0.7	0.3	0.02

In crude tobacco sap at the same dilution, both strains were inactivated in slightly more than 72 hours at room temperature. The dilution end point varied from 10^{-3} to 10^{-4} in different tests.

Both strains were transmitted from cucumber to tobacco and back to cucumber by *Aphis gossypii* and *Myzus persicae*. The virus was nonpersistent in the insect vectors.

Tobacco plants that had recovered from infection with either G.1B or T.2B were susceptible to reinfection with a strain of cucumber mosaic virus (CMV) isolated from gladiolus, but systemic symptoms induced by the challenging virus were both slower to appear and milder than those in check plants inoculated with CMV alone. Reciprocal experiments, using CMVP as challenging virus, were not attempted because of the indefiniteness of its symptoms on the local lesion hosts of CMV usually employed for such purposes. The two CMVP strains protected tobacco against each other but not against the tobacco ring spot virus nor, in some instances, against the tomato ring spot virus (Cornell strain). However, the cross protection tests with tomato ring spot virus, alfalfa mosaic virus, and the Doolittle strain of CMV were inconclusive because of low percentages of transmission of these viruses to check plants under the conditions of the experiment.

Both strains have been concentrated and partially purified several times from cucumber tissues by differential centrifugation (22). They differed from most of the stone-fruit virus isolates, however, in that appreciable amounts of virus came down with the pellets formed during the low speed runs. This loss of virus may have been due either to the relatively loose compaction of the pellets or to some adsorption of virus on other cell components in the extract. In spite of these losses, however, the end product, whether derived from fresh leaves, frozen leaves, or roots, was highly infective and contained "spherical" particles distributed about a prominent mode in the 30 mμ category (instrument calibration) containing at least half of the particles measured (Table III).

TABLE III

OBSERVED PARTICLE SIZE DISTRIBUTIONS IN EXTRACTS OF CUCUMBER TISSUE, HEALTHY AND INFECTED WITH G.1B

Source	Frequency per 1000					Number measured
	20 mμ	25 mμ	30 mμ	35 mμ	40 mμ	
Healthy fresh leaves (1)	228	199	240	133	200	736
Healthy fresh leaves (2)	264	165	240	157	174	743
Fresh leaves, G.1B (1)	132	138	514	174	42	689
Fresh leaves, G.1B (2)	154	185	508	126	27	2977
Fresh leaves, G.1B (1 and 2)	150	176	509	135	30	3666
Frozen leaves G.1B	35	61	511	288	105	1180
Roots G.1B (Cycle 2)	47	131	562	228	32	1177
Roots G.1B (Cycle 4)	23	102	565	255	55	1850
G.1B (over all)	87	134	531	200	48	7873

NOTE: Mean diameter of particles in the virus extracts = 29.92 mμ (instrument calibration).

The data in Table III, after angular transformation, were submitted to analysis of variance to compare particle size distributions (*a*) in the healthy vs. virus-infected fresh leaf replicates and (*b*) in virus-infected leaf vs. root samples. Comparison of the *F* values obtained in the analyses (Table IV) indicates a very high level of significance in the category–source interaction between healthy and virus-infected sources but not between virus-infected leaf and root sources. It can therefore be concluded that most of the particles measured in the virus extracts were of virus origin and that "virus particles" were about 30 mμ in diameter (instrument calibration). Since a factor of 1.16 has to be applied to convert instrument calibration to polystyrene calibration (22) the mean diameter of the "virus particles" is approximately 35 mμ. The stone-fruit virus isolates G.1 and T.2, from which the CMVP strains were separated, were characterized by particles of the order of 55 mμ diameter (polystyrene calibration) in peach petal extracts. Particles of the same size were also associated with isolate G.1 partially purified from cucumber leaves.

TABLE IV

F VALUES DERIVED FROM ANALYSIS OF VARIANCE IN PARTICLE SIZE DISTRIBUTIONS IN:
(A) HEALTHY VS. VIRUS FRESH LEAF REPLICATES
(B) VIRUS-INFECTED LEAF VS. ROOT SAMPLES

		F values		
Source of variance	D.F.	(A) Healthy vs. virus	(B) Leaf vs. root	*F* for significance
Between categories (C)	4	75.13	33.06	5.99**
Between sources (S)	1	4.04	< 0.1	4.96*
C × S interaction	4	47.99	< 1.0	5.99**

NOTE: *F* values required for significance at 1% level **, at 5% level *. Error (replicates) with 10 degrees of freedom.

Discussion

On the basis of symptomatology in herbaceous hosts, CMVP resembles cucumber mosaic virus (CMV) in some respects (2, 9, 10, 17) and alfalfa mosaic virus (AMV) in others (1, 8, 23, 24). In many of the hosts it would also be difficult to distinguish with certainty between cucumber mosaic and alfalfa mosaic. CMV strains, however, form a complex group, varying considerably in symptom expression (2, 9) and virulence. CMVP differs from most of the known strains of CMV and from AMV in inducing mild symptoms in cowpea and petunia and few or none in zinnia, tomato, and snapdragon. Recovery or partial recovery in tobacco has been reported for cucumber mosaic (2, 9) but not for alfalfa mosaic.

CMVP also has certain physical properties in common with both CMV (2, 9, 17) and AMV (8, 23, 24), namely, thermal inactivation point, dilution end point, and resistance to aging. CMV, like CMVP, also has a small thermal coefficient (2) and is associated with spherical particles 35 mμ in diameter (12). AMV, on the other hand, is reported to have particles only 16.5 mμ in diameter (11).

The information in hand regarding both physical properties and symptom expression strongly suggests that the CMVP isolates are atypical members of the CMV group. On the whole, they are less virulent than other known strains of CMV. The failure of the two isolates to give more than partial protection against another CMV strain indicates a more remote relationship than usual between CMV strains. In this respect CMVP resembles the strain of CMV isolated from barberry by Wilkinson (19).

There are several reasons for considering that CMVP is not implicated in the etiology of the diseases comprising the cherry-yellows–necrotic-ring-spot group. In the first place, all the cases of these viroses that have so far been examined have been associated with virus entities varying somewhat in characteristics, but causing severe dwarfing of cucumber plants. This dwarfing is accompanied either by suppression of terminal growth or by death of the growing point and, under some circumstances, of the whole plant (5, 20). Moreover, these virus entities either have not been transmitted to or have not caused symptoms in tobacco and other herbaceous hosts outside the Curcurbitaceae (4). On the other hand, CMVP, which differs from the other stone-fruit viruses in host range and symptom expression, has been associated with comparatively few cases of stone-fruit viroses. That it has been isolated relatively infrequently from trees shown to have been carrying it would suggest that it was either present in low concentration or not completely systemic in the *Prunus* hosts, or both. So far as could be ascertained, the CMVP strains neither induced distinctive symptoms in the *Prunus* hosts nor modified the symptoms caused by other stone-fruit viruses.

Acknowledgment

The authors wish to acknowledge with gratitude their indebtedness to J. H. H. Phillips for supplying information concerning the insect vectors and to J. D. Ferguson for his contribution to the electron microscopy.

References

1. Berkeley, G. H. A strain of the alfalfa mosaic virus on pepper in Ontario. Phytopathology, **37**, 781–789 (1947).
2. Bhargava, K. S. Some properties of four strains of cucumber mosaic virus. Ann. Appl. Biol. **38**, 377–388 (1951).
3. Boyle, J. S., Moore, J. D., and Keitt, G. W. Cucumber as a plant host in stone-fruit virus research. Phytopathology, **44**, 303–312 (1954).
4. Fulton, R. W. Comparative host ranges of two Prunus viruses. Phytopathology, **46**, 12 (1956).
5. Heinis, J. L. Correlation of ring spot virus reactions on stone-fruits and cucumber. Phytopathology, **46**, 163–167 (1956).
6. McWhorter, F. F. The utility of flower tissues for making inocula for difficult virus isolations. Phytopathology, **43**, 479 (1953).
7. Milbrath, J. A. Transmission of components of the stone-fruit latent virus complex to cowpea and cucumber from cherry flower petals. Phytopathology, **43**, 479 (1953).
8. Pierce, W. H. Viroses of the bean. Phytopathology, **24**, 87–115 (1934).
9. Price, W. C. Isolation and study of some yellow strains of cucumber mosaic. Phytopathology, **24**, 743–761 (1934).
10. Price, W. C. Classification of southern celery mosaic virus. Phytopathology, **25**, 947–954 (1935).
11. Ross, A. F. Purification and properties of alfalfa mosaic virus protein. Phytopathology, **31**, 394–410 (1941).

12. SILL, W. H., JR., BURGER, W. C., STAHMANN, M. A., and WALKER, J. C. Electron microscopy of cucumber virus 1. Phytopathology, **42**, 420–423 (1952).
13. VARNEY, E. H. and MOORE, J. D. Mechanical transmission of a virus from mosaic-infected elm to herbaceous plants. Phytopathology, **42**, 22 (1952).
14. VARNEY, E. H. and MOORE, J. D. A strain of tomato ring spot from American elm. Phytopathology, **42**, 477 (1952).
15. VARNEY, E. H. and MOORE, J. D. Tobacco and zinnia—two new herbaceous hosts for Prunus virus. Phytopathology, **44**, 509 (1954).
16. WEINTRAUB, M. and WILLISON, R. S. Studies on stone fruit viruses in cucurbit hosts. V. Thermal inactivation of some isolates. Phytopathology, **44**, 538–542 (1954).
17. WELLMAN, F. L. Identification of celery virus 1, cause of southern celery mosaic. Phytopathology, **34**, 695–725 (1934).
18. WILKINSON, R. E. Woody plant hosts of tobacco ring spot virus. Phytopathology, **42**, 478 (1952).
19. WILKINSON, R. E. *Berberis thunbergii*—A host for cucumber mosaic virus (*Marmor cucumeris*). Phytopathology, **43**, 489 (1953).
20. WILLISON, R. S. The effect of some stone fruit viruses on cucumber. Plant Disease Reptr. **35**, 254–255 (1951).
21. WILLISON, R. S. and WEINTRAUB, M. Studies on stone fruit viruses in cucurbit hosts. I. A method of evaluating the infectivity of infective juice. Phytopathology, **43**, 324–328 (1953).
22. WILLISON, R. S., WEINTRAUB, M., and FERGUSON, J. D. Purification and electron microscopy of viruses causing cherry-yellows and related diseases. Can. J. Botany, **34**, 86–103 (1956).
23. ZAUMEYER, W. J. A streak disease of peas and its relation to several strains of alfalfa mosaic virus. J. Agr. Research, **56**, 747–772 (1938).
24. ZAUMEYER, W. J. and WADE, B. L. The relationship of certain legume mosaics to bean. J. Agr. Research, **51**, 715–749 (1938).

STUDIES IN PLANT MINERAL NUTRITION

III. THE EFFECTS OF CALCIUM CONCENTRATION IN CULTURE SOLUTIONS UPON THE ABSORPTION OF IRON AND MANGANESE BY DWARF KIDNEY BEAN[1]

C. D. Taper and W. Leach

Abstract

Definite relationships between the absorption of iron, manganese, and calcium were revealed in experiments with dwarf kidney beans grown in complete nutrient solutions containing various concentrations of iron and manganese, and two concentrations of calcium. The effects of these treatments upon the leaf contents of iron and manganese were determined by spectrophotometric analysis.

It was found that increase in the concentration of either one of the metals, iron or manganese, relative to the concentration of the other, in the culture solution, appeared to depress the concentration of the other in the leaves, regardless of calcium level in the solution. Further, increase in the calcium level appeared to depress the accumulation by the leaves of both iron and manganese, regardless of their concentrations in the culture solution and the ratio between them.

The iron to manganese concentration ratio in the culture solution was a factor in determining the presence or absence of deficiency symptoms. With 42 p.p.m. of calcium in the culture solution, healthy plants resulted within an optimum iron : manganese ratio range of approximately 0.5 to 5.0, whereas, in the case of 143 p.p.m. of calcium, healthy plants resulted only when the ratio was 2, indicating a narrower optimum range for the higher calcium level. Iron: manganese ratios below the optimum range resulted in iron deficiency symptoms, and above this range, in manganese deficiency symptoms.

The data suggest that a minimum content of each of the metals, iron and manganese, must be present in the leaf tissues in order that healthy plants may result.

Introduction

In an earlier study (2) carried out by the authors with dwarf kidney bean, *Phaseolus vulgaris* var. *humilis*, grown in water culture solutions which gave a wide range of variations in iron and manganese supply and of iron: manganese ratios, the incidence of iron and manganese deficiency symptoms was correlated with two factors associated with the iron and manganese in the culture solutions. In the first place the actual concentrations of the two metals in the solutions were effective to the extent that when these concentrations were sufficiently low, in the case of either or both of these metals, corresponding deficiency symptoms appeared in the experimental plants. The second factor was the ratio of iron to that of manganese in the culture solution. It appeared that, providing a minimum amount of each of these metals is present, the iron:manganese ratio in the culture solution must lie between approximately 1.5 and 3.0 if healthy plants are to result. Iron:manganese ratios below this range resulted in iron deficiency symptoms, and above this range resulted in manganese deficiency symptoms, despite the fact that, in some instances, the actual solution concentrations of the metals were relatively high.

[1]Manuscript received April 4, 1957.
Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Macdonald College P.O., Que. With financial assistance from the National Research Council of Canada.

Analytical data suggested that the absolute amounts of iron and manganese in the leaves of the plants were related to the production of deficiency symptoms. It appeared that a necessary minimum content of each of these metals must be present in the leaves if healthy plants are to result. Further, a mutually depressant effect of either one of these metals, iron or manganese, on the absorption of the other was evidenced. Increase in the concentration of either one of these metals in the culture solution, relative to the concentration of the other, appeared to depress the accumulation of the other in the leaves.

It is evident that the investigation referred to above (2) revealed a definite relationship between the absorption of iron and manganese. The present study was undertaken to furnish additional data.

Methods

Dwarf kidney bean plants, *Phaseolus vulgaris* var. *humilis*, were grown (three to a 600 ml. beaker) in solutions which gave a wide range of variations in iron and manganese supply and of iron:manganese ratios, and two concentrations of calcium. The composition of the culture solutions is given in Tables I and II.

The culture solutions used were modifications and improvements of two culture solutions devised by Swanback (5) in an effort to obtain solutions

TABLE I

COMPOSITION OF CULTURE SOLUTIONS

Salt	Low calcium solution, g. per liter	High calcium solution, g. per liter
$Ca(NO_3)_2.4H_2O$	0.250	0.847
$NaH_2PO_4.H_2O$	0.078	0.078
KNO_3	0.214	—
$NaNO_3$	0.037	—
$Mg(NO_3)_2.6H_2O$	0.320	—
$MgSO_4.7H_2O$	0.308	0.616
K_2SO_4	—	0.185
$Na_2SO_4.10H_2O$	—	0.070

TABLE II

CONCENTRATIONS OF ELEMENTS IN THE CULTURE SOLUTIONS

Element	Low calcium solution, concentration, p.p.m.	High calcium solution, concentration, p.p.m.
Calcium	42.0	143.0
Nitrogen	99.6	100.0
Potassium	82.0	82.0
Magnesium	60.0	60.0
Sodium	22.9	22.9
Phosphorus	17.6	17.6
Sulphur	40.0	120.0

differing only in their calcium content. Table II shows that the modified solutions attained this aim completely in respect of cations and differed only in the case of the sulphate anion, SO_4^{--}. The effect of this upon the cations under study, namely, iron and manganese, was considered to be negligible. The solutions were renewed daily, however, in an effort to maintain a relatively constant pH.

Iron and manganese were added to the culture solutions as ferrous sulphate ($FeSO_4.7H_2O$) and manganese sulphate ($MnSO_4.4H_2O$) respectively. Other minor elements were added in the following concentrations: boron, 0.5 p.p.m.; zinc, 0.05 p.p.m.; molybdenum, 0.05 p.p.m.; and copper, 0.02 p.p.m.

The analytical procedures and water culture methods used were essentially those described by Leach, Bulman, and Kroeker (1). The iron and manganese content of leaf material was determined by spectrophotometric analysis. Analyses for soluble iron and manganese were carried out on acetone extracts, as described by Sideris and Young (3).

The characteristic symptoms of iron and manganese deficiencies as shown by dwarf bean were determined during the course of the preceding studies (2).

Experimental Results

The results of a group of water culture experiments with dwarf kidney bean are summarized in Table III.

An examination of Table III reveals a relationship between the iron and manganese content of the leaves of the experimental plants and the concentration of calcium in the culture solution. Increase in the concentration of calcium in the culture solution appeared to depress the accumulation by the leaves of both iron and manganese, regardless of their concentrations in the culture solution and the ratio between them.

Further, it will be seen that a mutually depressant effect of either one of the metals, iron or manganese, on the absorption of the other, is clearly in

TABLE III

EFFECTS OF CALCIUM CONCENTRATION IN THE CULTURE SOLUTION UPON THE ABSORPTION OF IRON AND MANGANESE BY DWARF BEANS

Culture No.	Culture solutions: Ca p.p.m.	Fe p.p.m.	Mn p.p.m.	Fe/Mn	Symptoms*	Iron and manganese content of leaves, mg. per g. dry material: Fe soluble	Fe total	Mn soluble	Mn total	Sol. Fe / Sol. Mn	Total Fe / Total Mn
1	42	5	25	0.2	−Fe	0.0579	0.1501	0.2193	0.6080	0.26	0.25
2	42	5	10	0.5	−Fe N	0.0843	0.2295	0.1394	0.3696	0.60	0.62
3	42	5	5	1.0	N	0.0970	0.2560	0.1238	0.2640	0.78	0.97
4	42	10	5	2.0	N	0.1402	0.3449	0.0968	0.2133	1.45	1.62
5	42	25	5	5.0	N	0.1782	0.6337	0.0479	0.1653	3.72	3.83
6	143	5	25	0.2	−Fe	0.0374	0.0999	0.1405	0.4590	0.27	0.22
7	143	5	10	0.5	−Fe	0.0490	0.1505	0.1048	0.3309	0.47	0.45
8	143	5	5	1.0	−Fe	0.0679	0.1939	0.0800	0.2337	0.85	0.83
9	143	10	5	2.0	N	0.1116	0.2845	0.0520	0.1943	2.15	1.46
10	143	25	5	5.0	−Mn	0.1332	0.4509	0.0225	0.1179	5.92	3.82

*N, normal; −Fe, iron deficiency; −Mn, manganese deficiency. Combinations of these indicate varying results within a treatment.

evidence. Increase in the concentration of either one in the culture solution, relative to the concentration of the other, appeared to depress the accumulation of the other in the leaves, regardless of calcium level in the solution.

It will again be seen, as in the previous study (2), that the iron:manganese concentration ratio in the culture solutions is an important factor in determining the presence or absence of deficiency symptoms. It has already been pointed out, however, that an increase in the calcium content of the culture solutions appeared to depress both iron and manganese absorption. This seemed to result in a narrowing of the range of tolerance in the iron:manganese concentration ratio within which healthy plants resulted. With 42 p.p.m. of calcium in the culture solutions the optimum range appeared to extend from 0.5 to 5.0+; whereas, in the case of 143 p.p.m. of calcium, healthy plants resulted only when the iron:manganese ratio was 2, indicating a narrower optimum range for the higher calcium level. It is apparent that the level of calcium in the culture solution is a factor in determining the optimum range of the iron:manganese ratio.

As in the previous experiments (2), iron:manganese ratios below this range resulted in iron deficiency symptoms, and above this range resulted in manganese deficiency symptoms.

Somers and Shive (4), working with soybean, found that the optimum range in the iron:manganese ratio was also shown in the contents of soluble iron and manganese in the leaves of their experimental plants. Table III shows that, unlike what was found with dwarf bean in the earlier experiments (2), there was a fair correspondence between the iron:manganese ratios in the culture solutions and the iron:manganese ratios (in respect of both soluble and total iron and manganese) in the leaves. In the case of the low calcium solution the total iron:total manganese ratios showed a somewhat closer correspondence than did the ratios derived from the analytical data of acetone extracts of leaf material. In the case of the high calcium solution the reverse was true, and the data derived from the data of acetone extracts approached very closely the ratios in the culture solution.

Discussion

A definite relationship between the absorption of iron, manganese, and calcium was exhibited in these experiments. The results uphold the view that the concentration ratios between iron and manganese in the culture solutions are related to the production of iron and manganese deficiency symptoms in that the ratios exhibit effects upon the total amounts of these metals which may be absorbed. Further, the data suggest that, providing the concentrations of these two metals in the solutions remain constant, the effects of a particular iron:manganese concentration ratio upon the total leaf content of these metals is modified by an increase in the calcium content of the culture solution. This results in a depression in the absorption of both iron and manganese, irrespective of the iron:manganese ratio.

Somers and Shive (4) proposed an oxidation–reduction hypothesis to account for the relationship which appears to exist between iron and manganese in plant metabolism. They considered that the functional iron in plant cells is present in the soluble ferrous state, and that this iron may be oxidized by manganese to the inactive and relatively insoluble ferric form; the result, with manganese in excess, being a manganese toxicity. They postulated that the reversal of this process results in an iron toxicity corresponding to a manganese deficiency. They concluded that, for healthy development of soybean, the ratio of soluble iron to soluble manganese in the leaf tissues must lie between 1.5 and 2.5.

The data derived from the present experiments demonstrate that the supposedly optimum range in the iron:manganese ratio, either in the culture solution or in the leaf tissues, is not invariable, but is changed by a change in the calcium level of the culture solution. Thus, the analytical data presented here do not furnish any very definite support for the hypothesis of Somers and Shive (4). On the contrary, the figures given in Table III suggest that a minimum content of each of the metals, iron and manganese, must be present in the leaf tissues in order that healthy plants may result.

It has been noted that the optimum range in the iron:manganese concentration ratio in the culture solution was narrower in the case of 143 p.p.m. of calcium in the solution than in the case of 42 p.p.m. of calcium. The figures representing the iron and manganese content of leaves in Table III appear to afford an explanation. They suggest that a depression of the iron and manganese content of the leaves below the quantities necessary for health resulted from an increase of the calcium concentration in the culture solution.

References

1. Leach, W., Bulman, R., and Kroeker, J. Studies in plant mineral nutrition. I. An investigation into the cause of gray speck disease of oats. Can. J. Botany, **32**, 358–368 (1954).
2. Leach, W., and Taper, C. D. Studies in plant mineral nutrition. II. The absorption of iron and manganese by dwarf kidney bean, tomato, and onion from culture solutions. Can. J. Botany, **32**, 561–570 (1954).
3. Sideris, C. P. and Young, H. Y. The growth and chemical composition of *Ananas comosus* in culture solutions with different iron to manganese ratios. Plant Physiol. **24**, 418–419 (1949).
4. Somers, L. I. and Shive, J. W. The iron manganese relation in plant metabolism. Plant Physiol. **17**, 582–602 (1942).
5. Swanback, T. R. Studies on antagonistic phenomena and cation absorption in the presence and absence of manganese and boron. Plant Physiol. **14**, 423–446 (1939).

CHROMOSOME NUMBERS OF CANADIAN WEEDS. I[1]

GERALD A. MULLIGAN[2]

Abstract

The chromosome numbers of 58 Canadian weeds are reported. Counts obtained on material of *Ambrosia psilostachya* DC. and *Epilobium angustifolium* L. differ from those obtained by other workers. The chromosome numbers of the following species had not previously been reported: *Silene cserei* Baumg. $2n=24$; *Axyris amaranthoides* L. $2n=18$; *Erigeron philadelphicus* L. $2n=18$; *Lepidium ramosissimum* Nels. $2n=64$; *Dracocephalum parviflorum* Nutt. $2n=14$; *Rumex fennicus* Murb. $2n=40$; *R. occidentalis* Wats. $2n=ca.$ 140; *R. stenophyllus* Ledeb. $2n=60$. The significance of some of these chromosome numbers is discussed.

Introduction

Weeds share a distinction with many cultivated plants; they are of major importance to man and yet their biology is practically unknown. The extraordinary variability of weeds, coupled with their foreign and frequently obscure origins, have made them unattractive subjects for the biologist. Taxonomic investigations of weedy groups by means of traditional methods are particularly unsatisfying and unrewarding. Unfortunately, exact taxonomic knowledge must be obtained before other fundamental studies, of vital importance in the ever continuing battle with weeds, can be dealt with intelligently.

Cytological studies on weeds at this laboratory have already thrown light on special taxonomic problems. It was found that two species of *Carduus* were forming extensive hybrid populations (59), and that a misunderstanding existed as to the presence of hybrids in two *Centaurea* species (58). A paper on *Euphorbia cyparissias* (57) revealed an intriguing situation in that sterile plants were shown to have a somatic count of $2n=20$ and fertile plants $2n=40$. A correlation between the size of epidermal cells and the number of chromosomes permitted mapping of the distribution of fertile and sterile stands from herbarium sheets. The fertile plants form extensive infestations whereas the sterile ones, although more widespread, are of little consequence as weeds. These early successes suggested that a comprehensive cytological study of Canadian weeds was required, not only for the sake of reinforcing taxonomic investigations but also with the thought that a thorough survey would eventually permit consideration of the possible relations of ploidy to aggressiveness, life duration, habitat, and so forth.

Several hundreds of species have been studied since 1953 and eventually all species of agricultural importance together with closely related nonweedy species will be included. It is true that chromosome numbers of a large part

[1]Manuscript received April 29, 1957.
Contribution No. 1599 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2]Botany and Plant Pathology Division (Weed Investigations), Ottawa.

of these species have been published elsewhere but these numbers have rarely been established for Canadian material. Where several chromosome races exist in a single species, a more thorough coverage will be made to establish the actual races found in Canada and to reduce the possibility of missing unknown differences in number. This first list of chromosome numbers is limited to species that have received a thorough bibliographical study, and for which mature specimens are available as vouchers.

Material and Methods

Mature seeds were collected from weeds growing in their natural habitats or from herbarium sheets. In a few instances, live material was transferred from the field into the greenhouse. The seed was sown in the greenhouse and five plants from each lot of seed were established. Root tips were taken from each plant, placed in water, and then exposed to a temperature of 2°–4° C. for 2 to 3 hours. They were then fixed in Randolph's Nawaschin solution, embedded in paraffin, and sectioned in the usual manner. The slides were stained in crystal violet. Specimens of the plants counted were collected and placed in the herbarium of the Botany and Plant Pathology Laboratory, Department of Agriculture, Ottawa (DAO). All drawings were made with the aid of a camera lucida.

Results

A complete list of the weeds counted, and their chromosome numbers, is given in Table I.

TABLE I

CHROMOSOME NUMBERS OF 58 CANADIAN WEEDS AND REFERENCES TO KNOWN PREVIOUS COUNTS

Species counted	Author's count ($2n$)	Source of seed, collector, and author's collection number	Previous counts		References to previous counts
			n	$2n$	
Amaranthaceae					
Amaranthus albus L.	32	Ottawa, Ontario, C. Frankton (1434)	16		(24) U.S.A.
Boraginaceae					
Cynoglossum officinale L.	24	Lombardy, Ont., C.F. (1500)	12		(7) U.S.A. and Europe
			ca. 12		(83) Eur.
				24	(7) U.S.A. and Eur.
Echium vulgare L.	32	Monckland Station, Ont., I. J. Bassett (2109); Ottawa, G. A. Mulligan (2209)		16	(45) Eur.
			16		(83) and (45) Eur.; (7) U.S.A. and Eur.
				32	(45) Eur.; (7) U.S.A.
Lappula echinata Gilib.	48	Cache Creek, British Columbia, G.A.M. (2204); Dawson Creek, B.C., G.A.M. (2205)		*ca.* 48	(83) Eur.
Lithospermum officinale L.	28	Ottawa, C.F. (2140)	14		(83) Eur.; (7) U.S.A. and Eur.
				28	(7) U.S.A. and Eur.
Caryophyllaceae					
Lychnis alba Mill.	24	Ottawa, G.A.M. (1489)	12		(75) Eur.; many other counts of either $n=12$ or $2n=24$—U.S.A., Eur. and Asia
Saponaria vaccaria L.	30	Ottawa, G.A.M. (1481)	15		(5) and (15) Eur.
				30	(6), (51), and (15) Eur.
Silene cserei Baumg.	24	Medicine Hat, Alberta, E. H. Moss (1510)	None		
cucubalus Wibel	24	Ottawa, G.A.M. (1488); Midland, Ont., I.J.B. (1516)	12		(5), (20), (70), (15), and (54) Eur.
				24	(6), (49), and (54) Eur.

TABLE I (*Continued*)

CHROMOSOME NUMBERS OF 58 CANADIAN WEEDS AND REFERENCES TO KNOWN PREVIOUS COUNTS

Species counted	Author's count ($2n$)	Source of seed, collector, and author's collection number	Previous counts n	Previous counts $2n$	References to previous counts
Chenopodiaceae					
Atriplex rosea L.	18	Penticton, B.C., H. Rhodes (2111)		18	(98) Eur.
Axyris amaranthoides L.	18	Lethbridge, Alta., J. J. Sexsmith (1509)	None		
Chenopodium capitatum (L.) Aschers.	18	Ottawa, G.A.M. (1496)		18	(41) Eur.
Compositae					
Ambrosia artemisiifolia L.	36	Ottawa, G.A.M. (1474); Empress, Alta., E. H. Moss (2148)	14*		(62) Eur.
			18		(12) and (36) U.S.A.
var. *elatior* Desc.					
psilostachya DC. var. *coronopifolia* (T. & G.) Farw.	72	Espanola, Ont., I.J.B. (2210)	50–52		(24) U.S.A.
trifida L.	24	Ottawa, I.J.B. (1479)	12		(12) and (36) U.S.A.; (62) Eur.
Anthemis cotula L.	18	Upton, Quebec, C.F. (2115)		18	(99) and (22) Eur.
Artemisia vulgaris L.	16	Ottawa, G.A.M. (1439)	8		(9), (10), (38), and (101) Eur.
			9*		(93) Eur.
Erigeron canadensis L.	18	Briercrest, Saskatchewan, D. R. Lindsay (1524); Ladner, B.C., D. Faris (2121)	9		(65) Asia; (12) U.S.A.
philadelphicus L.	18	Ottawa, G.A.M. (1512)	None		
Hieracium aurantiacum L.	36	Iron Bridge, Ont., I.J.B. (1492)	*ca.* 18		(72) Eur.
			18		(8) Eur.
				ca. 36	(72) Eur.
				36	(74) Asia; (8) Eur.
Hypochaeris radicata L.	8	Port Coquitlam, B.C., D.F. (1525)	4		(60) Eur.
				8	(49) Eur.; (24) U.S.A.
Lactuca biennis (Moench) Fern.	34	White Rock, B.C., D.F. (2138)	17		(82) U.S.A.
				34	(2) U.S.A.
scariola L.	18	Hull, Que., I.J.B. (1460); Ottawa, C.F. (1461)	*ca.* 9		(18) U.S.A.
			9		(94) Eur.; (86) U.S.A.
				18	(2) and (24) U.S.A.
scariola L. forma *integrifolia* (Bogenh.) G. Beck	18	Ottawa, C.F. (1462)	9		(94) and (86) U.S.A.
Leontodon autumnalis L.	12	St. John, New Brunswick, G.A.M. (1497)	6		(60), (53), (61), (21), and (55) Eur.
				12	(4) and (68) Eur.
				24	(92) Eur.
Matricaria matricarioides (Less.) Porter	18	Ottawa, G.A.M. (1480)	9		(73) and (50) Eur.; (24) U.S.A.; (31) Eur.
				18	(99) and (92) Eur.; (84) Asia
Sonchus arvensis L.	54	Amherst, Nova Scotia, Mason (1453); Cloverdale, B.C., D.F. (2211); Bot. Gard., Antwerp, Belgium (2212); Bot. Gard., Brussels, Belgium (2229)		54	(79) Canada
			32*		(99) Eur.
				64*	(14) Eur.
arvensis L. var. *glabrescens* Guenth., Grab. & Wimm.	36	Midland, Ont., H. A. Senn (1450); Brandon, Manitoba, G. Stevenson (1455); Weyburn, Sask., C.F. (2213); Halvorgate, Sask., C.F. (2214); Lethbridge, Alta., Wagner (1507); Lethbridge, Alta., D.R.L. (2215); White Rock, B.C., D.F. (2216)		36	(79) Can.
arvensis × *S. arvensis* L. var. *glabrescens*	45	White Rock, B.C., D.F. (2217)			
asper (L.) Hill.	18	Ottawa, C.F. (1443)		18	(100) Eur.; (3) U.S.A.; (73) Eur.; (24) U.S.A.; (92) Eur.
oleraceus L.	32	Montreal, Que., Fr. J.P. Bernard (2206); Ottawa, G.A.M. (1442)	8		(53) Eur.
				16	(14) Eur.
			16		(28) Asia; (12) U.S.A.
				32	(3) U.S.A.; (73) Eur.; (24) U.S.A.
Tragopogon dubius Scop.	12	Sault Ste. Marie, Ont., I.J.B. (2123)	6		(66) U.S.A.
				12	(67) U.S.A. and Can.
orientalis L.	12	Williams Lake, B.C., G.A.M. (2185)		12	(67) Eur.
pratensis L.	12	Sault Ste. Marie, Ont., I.J.B. (2124)	6		(96) Eur.; (12) U.S.A.; (95) Eur.
				12	(95) Eur.; (67) Can., U.S.A., and Eur.
			7*		(25) Asia

*This count is questionable.

TABLE I (*Continued*)

CHROMOSOME NUMBERS OF 58 CANADIAN WEEDS AND REFERENCES TO KNOWN PREVIOUS COUNTS

Species counted	Author's count ($2n$)	Source of seed, collector, and author's collection number	Previous counts n	Previous counts $2n$	References to previous counts
Cruciferae					
Berteroa incana (L.) DC.	16	Fitzroy Harbour, Ont., G.A.M. (2125)	8		(30) Eur.
				16	(52) and (100) Eur.
Camelina microcarpa Andrz.	40	McLeod, Alta., D.R.L. (2108)		40	(52) and (89) Eur.
Capsella bursa-pastoris (L.) Medic.	32	Sandringham, Ont., I.J.B. (1493); Parry Sound, Ont., I.J.B. (1523)	16		(71) and (43) Eur.
				32	(26) U.S.A.; (91) Eur.; (78) U.S.A.; (54) Eur.
Erucastrum gallicum (Willd.) O. E. Schulz.	30	Carman, Man., D.R.L. (2128)		30	(52) Eur.
Erysimum cheiranthoides L.	16	Ottawa, C.F. (1444)	8		(30) Eur.; (80) U.S.A.
				16	(52) Eur.
Lepidium campestre (L.) R. Br.	16	Southwestern Ontario, I. L. Connors (1499); Log Valley, Sask., G. W. Selleck (2219)		16	(100) Eur.
densiflorum Schrad.	32	Ottawa, G.A.M. (1528)	16		(33) Eur.; (80) U.S.A.
				32	(52) Eur.
latifolium L.	24	Lethbridge, Alta., J.J.S. (2147)	*ca.* 24		(52) Eur.
perfoliatum L.	16	Lethbridge, Alta., D.R.L. (1527)	8		(32), (33), and (89) Eur.
				16	(52) Eur.
ramosissimum Nels.	64	Stirling, Alta., D.R.L. (2129)	None		
Neslia paniculata (L.) Desv.	14	Ottawa, G.A.M. (1485)	7		(30) Eur.
				14	(52) Eur.
Sisymbrium loeselii L.	14	Stand Off, Alta., D.R.L. (2130)	7		(33) Eur.
			7		(33), (91), and (54) Eur.
Thlaspi arvense L.	14	Ottawa, G.A.M. (1476); Lethbridge, Alta., D.R.L. (1495)		14	(52) and (49) Eur.
Hypericaceae					
Hypericum perforatum L.	32	Perth, Ont., D.R.L. (1529); Fruitvale, B.C., W. G. Dore (1472)	16		(63), (27), and (64) Eur.
				32	(64) and (96) Eur.
Labiatae					
Dracocephalum parviflorum Nutt.	14	Ottawa, G.A.M. (2131); French River, Ont., I.J.B. (2158); Briercrest, Sask., D.R.L. (2107)	None		
Leguminosae					
Medicago lupulina L.	16	Ottawa, G.A.M. (1490); Orillia, Ont., I.J.B. (1517)	8		(16) Can.; (11) U.S.A.; (37) Eur.
				16	(19) Eur.; (16) Can.; (90) Eur. (97) U.S.A.; (68) Eur. (90) Eur.
Melilotus alba Desr.	16	Ottawa, G.A.M. (1515)	8		(69) U.S.A.; many other counts
				32	of either $n=8$ or $2n=16$—U.S.A. and Eur.
Lythraceae					
Lythrum salicaria L.	60	Ottawa, C.F. (1440)	15		(76) and (77) Asia
				30	(85) Asia
			25		(87) Eur.
				50	(48) Eur.
				60	(42) Eur.
Onagraceae					
Epilobium angustifolium L.	72	Ironside, Que., G.A.M. (1503); Viceroy, Sask. C.F. (2146); Creston, B.C., G.A.M. (2144)	None		
Epilobium angustifolium L.	36	Bot. Gard., Hann. Munden, Germany (2145); Bot. Gard., Copenhagen, Denmark, and collected in Northern Iceland (2221); Bot. Gard., Moscow, U.S.S.R., and collected at Pushinsky (2220); Bot. Gard., Moscow, and collected in Baksan Gorge on the Caucasian Expedition (2192); Bot. Gard., Moscow (2190).	18		(56) Eur.
				36	(56) Eur.; (35) U.S.A. (Calif.); (50) Eur.

TABLE I (*Concluded*)

CHROMOSOME NUMBERS OF 58 CANADIAN WEEDS AND REFERENCES TO KNOWN PREVIOUS COUNTS

Species counted	Author's count ($2n$)	Source of seed, collector, and author's collection number	Previous counts n	Previous counts $2n$	References to previous counts
Polygonaceae					
Polygonum convolvulus L.	40	Ottawa, C.F. (1433)	10		(30) Eur.
				20	(30) Eur.
				40	(1) and (46) Eur.
hydropiper L.	20	Ottawa, C.F. (1504)		20	(31) and (48) Eur.
				22*	(77) Eur.
Rumex crispus L.	60	Ottawa, C.F. (2132); Crichton, Sask., A. C. Budd (2222)	30		(40) Asia; (29) Eur.; (39) Asia; (34) Can. and U.S.A.
				60	(47) Eur.
			32*		(13) U.S.A.
fennicus Murb.	40	Macdonald, Man., C.F. (2224); Meadow, Man., C.F. (2225); Gravelbourg, Sask., C.F. (2226); Macoun, Sask., C.F. (2227); Crichton, Sask., A.C.B. (2181); Conde, South Dakota, J. F. Brenckle (2228)	None		
occidentalis Wats.	*ca.* 140	Macdonald, Sask., C.F. (2223); Swift Current, Sask., A.C.B. (2183); Crichton, Sask., A.C.B. (2182)	None		
stenophyllus Ledeb.	60	Swift Current, Sask., A.C.B. (2136)	None		

*This count is questionable.

Discussion

Forty-eight of the species examined had previously been counted by other workers; however, only six of these counts were on Canadian material. This was to be expected, as it was precisely this lack of information on Canadian weeds that prompted this study. According to European and Asiatic workers, of the 48 species counted, five have two or more chromosome races. The five weeds (with the author's count and, in parentheses, the chromosome numbers obtained by other workers) are as follows: *Echium vulgare* 32 (16, 32); *Leontodon autumnalis* 12 (12, 24); *Medicago lupulina* 16 (16, 32); *Lythrum salicaria* 60 (30, 50, 60); *Polygonum convolvulus* 40 (20, 40). Apparently there has been no attempt to relate the differences in chromosome numbers to any morphological differences that might exist in any of these five species. Because there is no known way of separating these chromosome races morphologically and as a very limited amount of Canadian material has been examined, it is impossible to know whether only one chromosome race is found in Canada or even if the number obtained by the author is the common one. It is of interest to mention that the diploid counts on *Lythrum salicaria* by Shinke (76, 77) and Takahashi (85) are on Japanese material identified as *L. salicaria* L. var. *vulgare* DC. subvar. *genuina* Koehne. This suggests the possibility that the three levels of polyploidy in *Lythrum salicaria* may be linked up with morphological differences.

Canadian material of *Sonchus arvensis* from Nova Scotia and British Columbia was found by the author to have 54 somatic chromosomes. This number was first reported by Shumovich and Montgomery (79) on material from many locations in the Province of Ontario. Other counts on this species

by Wulff (99) and Erlandsson (14) were $n=32$ and $2n=64$ respectively. As suggested by Shumovich *et al.* (79) these counts appear to be in error. Mitotic counts by the author on plants grown from seed received from Antwerp and Brussels, Belgium, were all 54. Stebbins, Jenkins, and Walters (82) were the first to suspect that the chromosome count of $n=32$ reported by Wulff (99) was wrong. They wrote, "Wulff (1937) has reported the haploid number of $n=32$ for *S. arvensis*; but his drawing indicates that the chromosomes in this species are very difficult to count. In view of the fact that *S. palustris* and *S. javanicus* which are rather similar to *S. arvensis* in external morphology, both have numbers based on $x=9$, the possibility that the haploid number in *S. arvensis* is $n=36$ should be considered". The haploid number is $n=27$ not $n=36$ as suggested by Stebbins *et al.* (82). However, their hypothesis, coupled with the chromosome counts of Shumovich *et al.* (79) and of the author, indicates that *S. arvensis* has a chromosome count of $n=27$ and $2n=54$, not $n=32$ and $2n=64$.

New Counts

Of the following 10 species, eight have not been counted before whereas the other two had chromosome counts different from those obtained by other workers.

Silene cserei, $2n=24$ (Fig. 1), an introduced weed of Asiatic origin, has the common chromosome number of the genus *Silene*.

Axyris amaranthoides, $2n=18$ (Fig. 2) is also introduced and of Asiatic origin. This chromosome count is the first made on any member of the genus *Axyris* and like most other members of the family Chenopodiaceae, *A. amaranthoides* has a base number of $x=9$.

Ambrosia psilostachya var. *coronopifolia*, $2n=72$ (Fig. 3) is a North American native plant. The gametic numbers of the other three commonly recognized members of this genus, *A. trifida*, *A. bidentata*, and *A. artemisiifolia*, are 12, 17, and 18 respectively. The base number of *A. psilostachya* var. *coronopfolia* would be $x=18$, the same base number as *A. artemisiifolia*, the species that it most closely resembles morphologically. Heiser and Whitaker (24) counted $n=50$–52 chromosomes on material identified as *Ambrosia psilostachya* D.C. Their count coupled with the author's somatic count of 72 supports the view that there is more than one taxon in the *Ambrosia psilostachya* complex. The author has seen all the original descriptions and a limited amount of material of members of the *A. psilostachya* complex and it appears that at least three distinct taxa exist. There are many synonyms and some doubt among taxonomists whether these taxa should be considered species, subspecies, or varieties.

Erigeron philadelphicus, $2n=18$ (Fig. 4), is native to North America. Gaiser (17) lists chromosome counts of $n=8$ and $2n=16$ for this species, referring to Land (44). However, no counts on this species are given in Land's paper. The somatic count of 18 fits the base number of $x=9$ that is most common in this genus.

Lepidium ramosissimum, $2n=64$ (Fig. 5), is also native to North America. Most of the members of this genus have a base number of $x=8$. The highest number previously reported for this genus was $2n=32$. The author's count for *L. ramosissimum* is the same as that recorded by Manton (52) on *Lepidium draba* L. However, *L. draba* is considered by many botanists to belong to the genus *Cardaria*.

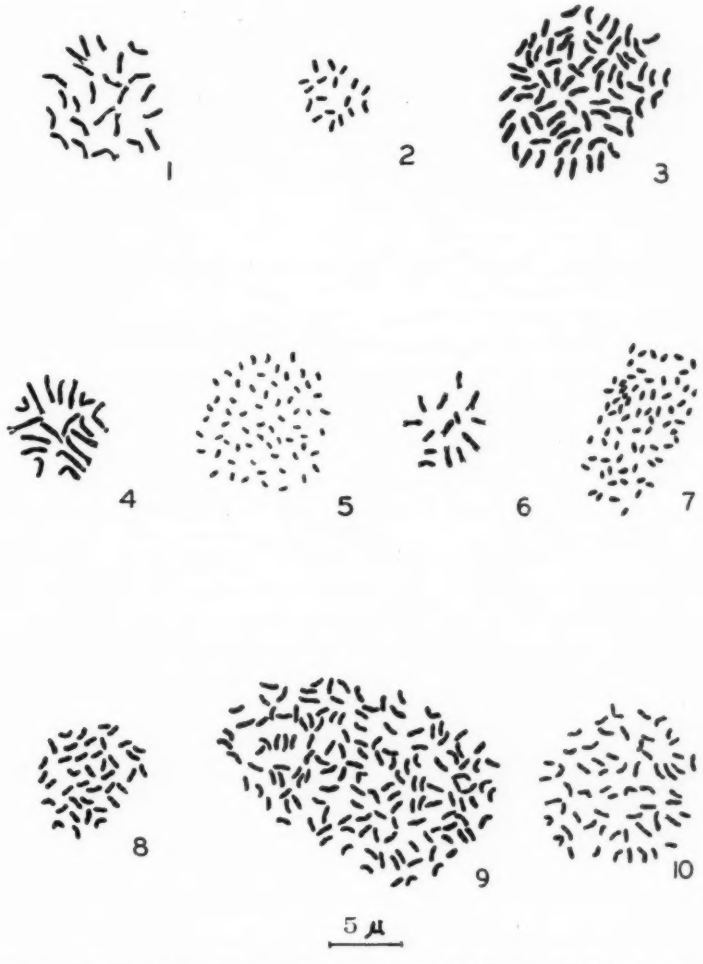


FIG. 1. *Silene cserei*, $2n=24$. FIG. 2. *Axyris amaranthoides*, $2n=18$. FIG. 3. *Ambrosia psilostachya* var. *coronopifolia*, $2n=72$. FIG. 4. *Erigeron philadelphicus*, $2n=18$. FIG. 5. *Lepidium ramosissimum*, $2n=64$. FIG. 6. *Dracocephalum parviflorum*, $2n=14$. FIG. 7. *Epilobium angustifolium*, $2n=72$. FIG. 8. *Rumex fennicus*, $2n=40$. FIG. 9. *R. occidentalis*, $2n=ca.$ 140. FIG. 10. *R. stenophyllus*, $2n=60$.

Dracocephalum parviflorum, $2n=14$ (Fig. 6) is also a North American native. Löve and Löve (49) and Sokolovskaja and Strelkova (81) counted the same number of chromosomes on material of *D. ruyschiana* L. and *D. altaicense* Laxm. respectively.

Epilobium angustifolium, $2n=72$ (Fig. 7), is a very variable circumboreal species. Plants of *Epilobium angustifolium* grown from seed collected in Quebec, Saskatchewan, and British Columbia had a somatic number of 72. Johansen (35) counted 36 somatic chromosomes on Californian material of this species. Plants of *E. angustifolium* grown from seed received from Germany, Denmark, and the U.S.S.R. had, in all cases, 36 mitotic chromosomes. The same chromosome count was obtained on European material by Löve and Löve (50) and Michaelis (56). No clear morphological differences were found between the tetraploid and octoploid *E. angustifolium*. Often where two chromosome races occur in one species, the race with the highest number of chromosomes has consistently larger pollen grains. However, no correlation between pollen size and chromosome number was found in the material counted. Crosses were made between plants from Creston, B.C. ($2n=72$) and Viceroy, Sask. ($2n=72$) and pods with a high percentage of apparently good seeds were obtained. These seeds germinated readily and the F_1 plants had 72 somatic chromosomes. Pollen from these F_1 plants appeared fertile when a stain of cotton blue in lactophenol was applied. The Creston and Viceroy plants differed markedly in the shape of the stamens but were completely compatible genetically. Further crosses were made between plants from Germany ($2n=36$) and Creston ($2n=72$), and also Viceroy ($2n=72$) and Germany ($2n=36$). As in the crosses between the Creston and Viceroy octoploids, a high percentage of apparently good seeds was obtained. These seeds germinated readily and the F_1 plants all had 54 somatic chromosomes and a low percentage of "fertile" pollen. It appears that the Canadian octoploids are rather closely related to the European tetraploids genetically. A cursory examination of DAO herbarium material failed to reveal any clear morphological differences between Canadian and European material of *E. angustifolium*. The status of North American material of this species would no doubt be clarified by establishing the chromosome numbers of material from many different locations in North America. However, an understanding of the *E. angustifolium* complex would best be obtained by applying the methods of experimental taxonomy to both North American and European material.

Rumex fennicus, $2n=40$ (Fig. 8), and *R. stenophyllus*, $2n=60$ (Fig. 10) are of Eurasiatic origin. Both of these introduced plants belong to the subgenus *Lapathum*; the members of this subgenus apparently have a base number of $x=10$.

Rumex occidentalis, $2n=ca.$ 140 (Fig. 9), is native to North America. The somatic count of 140 is the first at that polyploid level made on any members of the subgenus *Lapathum*. The gametic numbers now known in this subgenus are; 10, 20, 30, 40, 50, 60, 70, 80, and 100, an almost complete euploid series.

Acknowledgments

The writer is indebted to Drs. C. Frankton, R. J. Moore, and H. A. Senn, all of the Botany and Plant Pathology Laboratory, Science Service, Ottawa, for their interest and assistance throughout this study. I also express my gratitude to the many workers who were kind enough to obtain plant material for me.

References

1. Andersson *in* Löve, A. and Löve, D. (48).
2. Babcock, E. B., Stebbins, G. L., and Jenkins, J. A. Chromosomes and phylogeny in some genera of the Crepidinae. Cytologia, Fujii Jubilae i, 188–210 (1937).
3. Barber, H. N. Spontaneous hybrids between *Sonchus asper* and *S. oleraceus*. Ann. Botany, **5** (n.s.), 375–377 (1941).
4. Bergman, B. Zytologische Studien über die Fortpflanzung bei den Gattungen *Leontodon* und *Picris*. Svensk Botan. Tidskr. **29**, 155–301 (1935).
5. Blackburn, K. B. Chromosome number in *Silene* and the neighboring genera. Z. Induktive Abstammungsu. Vererbungslehre Suppl. **1**, 439–446 (1928).
6. Blackburn, K. B. and Boult, J. J. The status of the genus *Saponaria* and its near allies considered in the light of their cytology. Proc. Univ. Durham Phil. Soc. **8**, 260–266 (1930).
7. Britton, D. M. Cytogenetic studies on the Boraginaceae. Brittonia, **7**, 233–266 (1951).
8. Christoff, M. Die genetische Grundlage der apomiktischen Fortpflanzung bei *Hieracium aurantiacum* L. Z. Induktive Abstammungsu. Verebungslehre, **80**, 103–125 (1942).
9. Clausen, J., Keck, D. D., and Hiesey, W. M. Experimental taxonomy. Carnegie Inst. Wash. Year-book, **38**, 123–127 (1939).
10. Clausen, J., Keck, D. D., and Hiesey, W. M. Experimental studies on the nature of species. I. Effect of varied environments on western North American plants. Carnegie Inst. Wash. Publ. **520**, 1–452 (1940).
11. Cooper, D. C. Macrosporogenesis and embryology of *Medicago*. J. Agr. Research, **51**, 471–477 (1935).
12. Cooper, D. C. and Mahony, K. L. Cytological observations on certain Compositae. Am. J. Botany, **22**, 843–848 (1935).
13. Dudgeon, W. Morphology of *Rumex crispus*. Botan. Gaz. **5**, 393–420 (1918).
14. Erlandsson *in* Löve, A. and Löve, D. (48).
15. Favarger, C. Recherches caryologiques sur la sous-famille des Silénoidées. Ber. schweiz. botan. Ges. **56**, 364–466 (1946).
16. Fryer, J. R. Cytological studies in *Medicago*, *Melilotus* and *Trigonella*. Can. J. Research, **3**, 3–50 (1930).
17. Gaiser, L. O. A list of chromosome numbers in angiosperms. Genetica, **8**, 439 (1926).
18. Gates, R. R. and Rees, E. M. A cytological study of pollen development in *Lactuca*. Ann. Botany (London), **35**, 365–398 (1921).
19. Ghimpu, de M. V. Contribution à l'étude caryologique du genre *Medicago*. Compt. rend. **187**, 245–247 (1928).
20. Griesinger, R. Über hypo- und hyperdiploide Formen von *Petunia*, *Hyoscyamus*, *Lamium*, und einige andere Chromosomenzählugen. Ber. deut. botan. Ges. **55**, 262–269 (1937).
21. Hagerup, O. Nordiske Kromosomen—Tal. I. Dansk Botan. Tidsskr. **45**, 385–395 (1941).
22. Harling, G. Embryological studies in the Compositae. Part I. Anthemideae-Anthemidinae. Acta Horti Berg. **15**, 136–168 (1951).
23. Hartwich, H. *in* Tischler (88).
24. Heiser, C. B., Jr. and Whitaker, T. W. Chromosome number, polyploidy, and growth habit in California weeds. Am. J. Botany, **35**, 179–186 (1948).
25. Heitz, E. Der Nachweis der Chromosomen. Vergleichlende Studien über ihre Zahl, Gröbe und Form in Pflanzenreich I. Z. Botan. **18**, 625–681 (1926).
26. Hill, S. E. Chromosome numbers in the genus *Bursa*. Biol. Bull. Marine Biol. Lab. **53**, 413–415 (1927).
27. Hoar, C. S. and Haertl, E. J. Meiosis in the genus *Hypericum*. Botan. Gaz. **93**, 197–204 (1932).
28. Ishikawa, M. A list of the number of chromosomes. Botan. Gaz. (Tokyo), **30**, 404–448 (1916).
29. Jaretzky, R. Die Degenerationserscheinungen in den Blüten von *Rumex flexuosus* Forst. Jahrb. wiss. Botan. **66**, 301–320 (1927).

30. JARETZKY, R. Untersuchungen über Chromosomen und Phylogenie bei einigen Cruciferen. Jahrb. wiss. Botan. **68**, 1–45 (1928).
31. JARETZKY, R. Histologische und Karyologische Studien an Polygonaceen. Jahrb. wiss. Botan. **69**, 357–490 (1928).
32. JARETZKY, R. Die Chromosomenzahlen in der Gattung *Matthiola*. Ber. deut. botan. Ges. **47**, 82–85 (1929).
33. JARETZKY, R. Beiziehungen zwischen Chromosomenzahl und Systematic bei den Cruciferen. Jahrb. wiss. Botan., **76**, 485–527 (1932).
34. JENSEN, H. W. Meiosis in *Rumex*. I. Polyploidy and the origin of new species. Cytologia, **7**, 1–22 (1936).
35. JOHANSEN, D. A. New chromosome numbers in the Onagraceae. Am. J. Botany, **16**, 595–597 (1929).
36. JONES, K. L. Studies on Ambrosia: III. Pistillate *Ambrosia elatior* X *A. trifida* and its bearing on matroclinic sex inheritance. Botan. Gaz. **105**, 226–232 (1943).
37. KARPETCHENKO *in* Tischler (88).
38. KECK, D. D. A revision of the *Artemisia vulgaris* complex in North America. Proc. Calif. Acad. Sci. **25**, 421–468 (1946).
39. KIHARA, H. Über die Vorbehandlung einiger pflanzlicher Objekte bei der Fixierung der Pollenmutterzellen. Botan. Mag. (Tokyo), **41**, 124–128 (1927).
40. KIHARA, H. and ONO, T. *in* Tischler (88).
41. KJELIMARK, S. Einige neue Chromosomenzahlen in der Familie Chenopodiaceae. Botan. Notiser, 136–140 (1934).
42. LA COUR *in* DARLINGTON, C. D. and JANAKI, A. E. K. Chromosome atlas of cultivated plants. George Allen & Unwin Ltd., London. 1945.
43. LAIBACH, F. Zur Frag nach der Individualitat der Chromosomen in Pflazenreich. Beih. Botan. Centr. **22**, 191–210 (1907).
44. LAND, W. J. G. Double fertilization in Compositae. Botan. Gaz. **30**, 252–259 (1900).
45. DE LITARDIÈRE, R. Recherches caryologiques et Caryotaxonomiques sur les Boraginacées. II. Nombres chromosomiques dans le genre *Echium*. Boissiera, **7**, 155–165 (1943).
46. LÖVE, A. Polyploidy in *Polygonum convolvulus* L. Hereditas, **28**, 227–228 (1942).
47. LÖVE, A. Cytogenetic studies in *Rumex*. III. Some notes on the Scandinavian species of the genus. Hereditas, **28**, 289–296 (1942).
48. LÖVE, A. and LÖVE, D. Chromosome numbers of Scandinavian plants. Botan. Notiser, 19–59 (1942).
49. LÖVE, A. and LÖVE, D. Cyto-taxonomical studies on boreal plants. III. Some new chromosome numbers of Scandinavian plants. Arkiv Botan. **31A**, 1–22 (1944).
50. LÖVE, A. and LÖVE, D. Chromosome numbers of northern plant species. Reykjavik, 9–131 (1948).
51. LÖVE, D. Some contributions to the cytology of Silenoideae. Svensk botan. Tidskr. **36**, 262–270 (1942).
52. MANTON, I. Introduction to the general cytology of the Cruciferae. Ann. Botany (London), **46**, 509–556 (1932).
53. MARCHAL, E. Recherches sur les variations numériques des chromosomes dans la série végétale. Mém. acad. R. Belg. Ser. 2, **5**, 1–108 (1920).
54. MATTICK *in* Tischler (88).
55. MEYER, K. Über die Entwicklung des Pollens bei *Leontodon autumnalis* L. Ber. deut. botan. Ges. **43**, 108–114 (1925).
56. MICHAELIS, P. Zur Cytologie und Embryoentwicklung von *Epilobium*. Ber. deut. botan. Ges. **43**, 61–67 (1925).
57. MOORE, R. J. and LINDSAY, D. R. Fertility and polyploidy of *Euphorbia cyparissias* in Canada. Can. J. Botany, **31**, 152–163 (1953).
58. MOORE, R. J. and FRANKTON, C. Cytotaxonomy of three species of *Centaurea* adventive in Canada. Can. J. Botany, **32**, 182–186 (1954).
59. MOORE, R. J. and MULLIGAN, G. A. Natural hybridization between *Carduus acanthoides* and *Carduus nutans* in Ontario. Can. J. Botany, **34**, 71–85 (1956).
60. NAWASCHIN, M. *in* Tischler (88).
61. NEGODI, G. Contributo alla cariologia del genere *Hypochaeris* L. Atti. soc. nat. e mat. Modena, **67**, 13–14 (1936).
62. NEGODI, G. Nuovi reperti cariologici su Fanerogame. Atti. soc. nat. e mat. Modena, **68**, 9–11 (1937).
63. NIELSEN, N. Chromosome numbers in the genus *Hypericum*. Hereditas, **5**, 378–382 1924.
64. NOACK, K. L. Über Hypericum-Kreuzungen. VI. Fortpflanzungsverhältnisse und Bastarde von *Hypericum perforatum* L. Z. Induktive Abstammungsu. Vererbungslehre, **76**, 569–601 (1939).
65. OKABE, S. Regarding apomixis of a few wild seed plants of this country. Botan. Mag. (Tokyo), **48** (6) & (7) (1934).

66. OWNBEY, M. Natural hybridization and amphiploidy in the genus *Tragopogon*. Am. J. Botany, **37**, 487–499 (1950).
67. OWNBEY, M. and MCCOLLUM, G. D. The chromosomes of *Tragopogon*. Rhodora, **56**, 7–21 (1954).
68. POLYA, L. Chromosome numbers of certain alkali plants. Arch. Biol. Hung. **18**, 145–148 (1948).
69. ROGERS, W. E. Notes on *Melilotus alba*, white sweet clover. Proc. Iowa Acad. Sci. **24**, 415–423 (1917).
70. ROHWEDER, H. Weitere Beiträge zur Systematik und Phylogenie der Caryophyllaceen. Beih. Botan. Centr. **59**, 1–58 (1939).
71. ROSENBERG, O. Über die Individualität der Chromosomen in Pflanzenreich. Flora (Jena), **93**, 251–259 (1904).
72. ROSENBERG, O. Die reduktion Steilung und ihre Degeneration in *Hieracium*. Svensk Botan. Tidskr. **11**, 145–206 (1917).
73. RUTLAND, J. P. A list of chromosome numbers of British plants. New Phytologist, **40**, 210–214 (1941).
74. SAKAI, K. Studies on the chromosome number in alpine plants. II. Japan. J. Genetics, **11**, 68–73 (1935).
75. SCHURHOFF, P. N. Über die Teilung des generativen Kerns vor der Keimung des Pollenkorns. Arch. Zellforsch. **15**, 145–159 (1919).
76. SHINKE, N. Chromosome arrangement. IV. The meiotic divisions in pollen mother-cells of *Sagittaria aginashi* Makino and *Lythrum salicaria* L. var. *vulgare* DC. subvar. *genuina* Koehne. Mem. Coll. Sci. Univ. Kyoto Ser. B, **4**, 283–308 (1929).
77. SHINKE, N. Spiral structure of chromosomes in meiosis in *Sagittaria aginashi*. Mem. Coll. Sci. Univ. Kyoto Ser. B, **9**, 367–392 (1934).
78. SHULL, G. H. *in* Tischler (88).
79. SHUMOVICH, W. and MONTGOMERY, F. H. The perennial sowthistles in northeastern North America. Can. J. Agr. Sci. **35**, 601–605 (1955).
80. SMITH, F. H. Some chromosome numbers in the Cruciferae. Am. J. Botany, **25**, 220–221 (1938).
81. SOKOLVSKAJA, A. P. and STRELKOVA, O. S. Polyploidy in the high mountain region of Pamir and Altai. Compt. rend. acad. sci. U.R.S.S. **21**, 68–71 (1938).
82. STEBBINS, G. L., JENKINS, J. A., and WALTERS, M. S. Chromosomes and phylogeny in the Compositae, Tribe Cichorieae. Univ. Calif. Publs. Botany, **26**, 401–430 (1953).
83. STREY, M. Karyologische Studien an Borraginoideae. Planta, **14**, 682–730 (1931).
84. SUZUKA, O. Chromosome numbers in pharmaceutical plants I. Repts. Inst. Biol. Research, **4**, 57–58 (1950).
85. TAKAHASHI *in* KIHARA, H., YAMAMOTO, Y., and HOSONO, S. A list of chromosome numbers of plants cultivated in Japan Shokubutsu Senshokutaisûno kenkyû, 195–327 (931).
86. THOMPSON, R. C., WHITAKER, T. W., and KOSAR, W. F. Interspecific genetic relationships in *Lactuca*. J. Agr. Research, **63**, 91–107 (1941).
87. TISCHLER, G. Revisionen früherer Chromosomenzählungen und anschliessende Untersuchungen. Planta, **8**, 685–697 (1929).
88. TISCHLER, G. Die Chromosomenzählen der Gefässpflanzen Mitteleuropas. Dr. W. Junk, 's-Gravenhage. 1950.
89. TITOVA, N. N. *in* Tischler (88).
90. TSCHECHOW, W. Karyo-systematical analysis of the tribe Trifolieae D.C. (Fam. Leguminosae). Bull. Appl. Botany Genet. and Plant Breeding (U.S.S.R.), Ser. II, **I**, 119–146 (1933).
91. VAARAMA, A. Beobachtungen uber die Meiose bei einigen Anthropochoren. Hereditas, **29**, 191–193 (1943).
92. VAARAMA *in* Löve, A. and Löve, D. (50).
93. WEINEDEL-LIEBAU, F. Zytologische Untersuchungen an *Artemisia*-Arten. Jahrb. wiss. Bot. **69**, 636–686 (1928).
94. WHITAKER, T. W. and JAGGER, I. C. Cytogenetic observations in *Lactuca*. J. Agr. Research, **58**, 297–306 (1939).
95. WINGE, O. Inheritance of species character in *Tragopogon*. A cytogenetic investigation. Compt. rend. trav. lab. Carlsberg, Sér. physiol. **22**, 155–193 (1938).
96. WINGE, O. *in* Tischler (88).
97. WIPF, L. Chromosome numbers in the root nodules and root tips of certain Leguminosae. Botan. Gaz. **101**, 51–67 (1939).
98. WULFF, H. D. Die Polysomatie der Chenopodiaceen. Planta, **26**, 275–290 (1936).
99. WULFF, H. D. Chromosomenstudien an der schleswig-holsteinischen Angiospermenflora I. Ber. deut. botan. Ges. **55**, 262–269 (1937).
100. WULFF, H. D. Chromosomenstudien an der schleswig-holsteinischen Angiospermenflora IV. Ber. deut. botan. Ges. **57**, 424–431 (1939).
101. WULFF, H. D. *in* Tischler (88).

NOTES

FINE STRUCTURE OF GUARD-CELL WALLS IN AVENA COLEOPTILE[1]

GEORGE SETTERFIELD

Guard cells commonly possess differentially thickened cell walls which are important in the opening and closing of stomata. Mühlethaler (6) has reported that the thickened walls of guard cells in hyacinth possess a multilayered structure although the arrangement of cellulose microfibrils is not visible in the picture he presents. Recently, during the course of electron-microscope studies on cell wall growth in *Avena* coleoptiles, transverse thin sections passing through guard cells were obtained. This report describes the fine structure of the thickened cell walls as revealed in these sections.

Studies were carried out on coleoptiles of Lanark variety oats. To determine the general structural features of the guard cells, outer epidermis was stripped from coleoptiles of various lengths, mounted in water, and observed in the light microscope. Stomata were found localized in narrow strips opposite the vascular bundles, as reported by Avery (1) for corn coleoptile. Fig. 1 shows a closed stoma (the term stoma is used here, as by Esau (3), to include the guard cells and pore between them) from a 40 mm. long coleoptile. The general structure of the guard cells (*G*) is that common to the Gramineae (3) and described in detail by Flint and Moreland (4) for sugar cane. The cells are narrow and elongated. They possess a thick-walled central region which maintains an approximately constant diameter during opening and closing of the stoma and thin-walled ends which swell when the stoma is open. A relatively thin-walled accessory cell (*A*) adjoins each guard cell.

Thin sections were obtained from an isolated coleoptile segment grown for 20 hours in a medium containing 0.01 *M* phosphate–citrate buffer (pH 5.0), 2% sucrose, and 8 p.p.m. indoleacetic acid (7). It had almost reached maximum elongation. The tissue was treated with pectinase, fixed, embedded, and sectioned by methods described previously (2, 9). Embedding medium was removed from the sections and they were shadowed with palladium–gold.

Fig. 2 is a low magnification electron micrograph of a transverse section through a stoma. The section has presumably passed through the central region of the guard cells (*G*) for both inner and outer walls are markedly thickened (in all electron micrographs the unlabelled arrows point toward the inside of the coleoptile). All walls of the accessory cells (*A*) on the other hand are thin. The remains of the cuticle (*C*) may be seen to pass over the entire surface of the guard cells into the air space. Collapsed cytoplasm (*Cy*) is present in the lumens of the guard cells.

[1]Issued as N.R.C. No. 4416.

Fig. 3 is a higher magnification picture of a region of the outer wall of one of the guard cells as indicated by the rectangle in Fig. 2. It can be seen that the thick wall is composed of fairly regular layers of cellulose microfibrils. These microfibrils appear as short segments in transverse section indicating that their orientation is longitudinal with respect to the cell axis. Some of the layers have fallen over during removal of embedding medium (*F*) while others have remained erect (*E*). The thickened inner wall possesses a similar structure. The regions between the layers of microfibrils presumably contained amorphous non-cellulosic wall material, most of which has been removed by pectinase treatment. (This material cannot be definitely identified as pectic owing to the crudeness of the pectinase preparation.)

This multilayered structure is very similar to that described by Bayley *et al.* (2) for the outer walls of regular epidermal cells. The striking similarity is clearly shown in Fig. 4, which is a transverse section (pectinase treated) of an outer epidermal wall near a cell junction, in a 10 mm. long coleoptile.

These observations show that although guard cells of *Avena* coleoptile possess different form and function as compared to regular epidermal cells, the thickened wall structure is similar in both and presumably arises by a similar mechanism. Furthermore, the guard cells elongate: in coleoptiles 10 mm. long, guard cells were about 45 μ long while in 30 mm. coleoptiles they had reached 80 μ. These walls would appear to be another example of the type designated "thickened primary" (5, 9) for which, as pointed out previously (2, 9), the mechanisms of deposition and extension are not completely clear. The multi-net model (8) which has been used to explain growth of thin parenchyma walls in coleoptiles (9, 11) would not seem to be entirely adequate.

A further point of interest is that the guard and accessory cells in a 30 mm. long coleoptile reach only about one-fifth the length of immediately adjacent epidermal cells. To compensate for this difference in cell length, slippage may occur between the accessory cells and adjacent epidermal cells. Alternatively, the regions of the individual epidermal cells adjacent to accessory cells may elongate less than the remaining parts of the same cells. Such differential intracellular elongation would be similar to that found in grass roots by Sinnott and Bloch (10).

1. Avery, G. S. Comparative anatomy and morphology of embryos and seedlings of maize, oats, and wheat. Botan. Gaz. **89**, 1–39 (1930).
2. Bayley, S. T., Colvin, J. R., Cooper, F. P., and Martin-Smith, C. A. The structure of the primary epidermal cell wall of *Avena* coleoptiles. J. Biophys. Biochem. Cytol. **3**, 171–182 (1957).
3. Esau, K. Plant anatomy. John Wiley and Sons, Inc., New York. 1953.
4. Flint, L. H. and Moreland, C. F. A study of the stomate in sugar cane. Am. J. Botany, **33**, 80–82 (1946).
5. Majumdar, G. P. and Preston, R. D. The fine structure of collenchyma cells in *Heracleum Sphondylium L.* Proc. Roy. Soc. (London). B, **130**, 201–217 (1941).
6. Mühlethaler, K. Elektronenmikroskopische Untersuchungen an pflanzlichen Geweben. Z. Zellforsch. u. mikroskop. Anat. **38**, 299–327 (1953).
7. Nitsch, J. P. and Nitsch, C. Studies on the growth of coleoptile and first internode sections. A new, sensitive straight-growth test for auxins. Plant Physiol. **31**, 94-111 (1956).

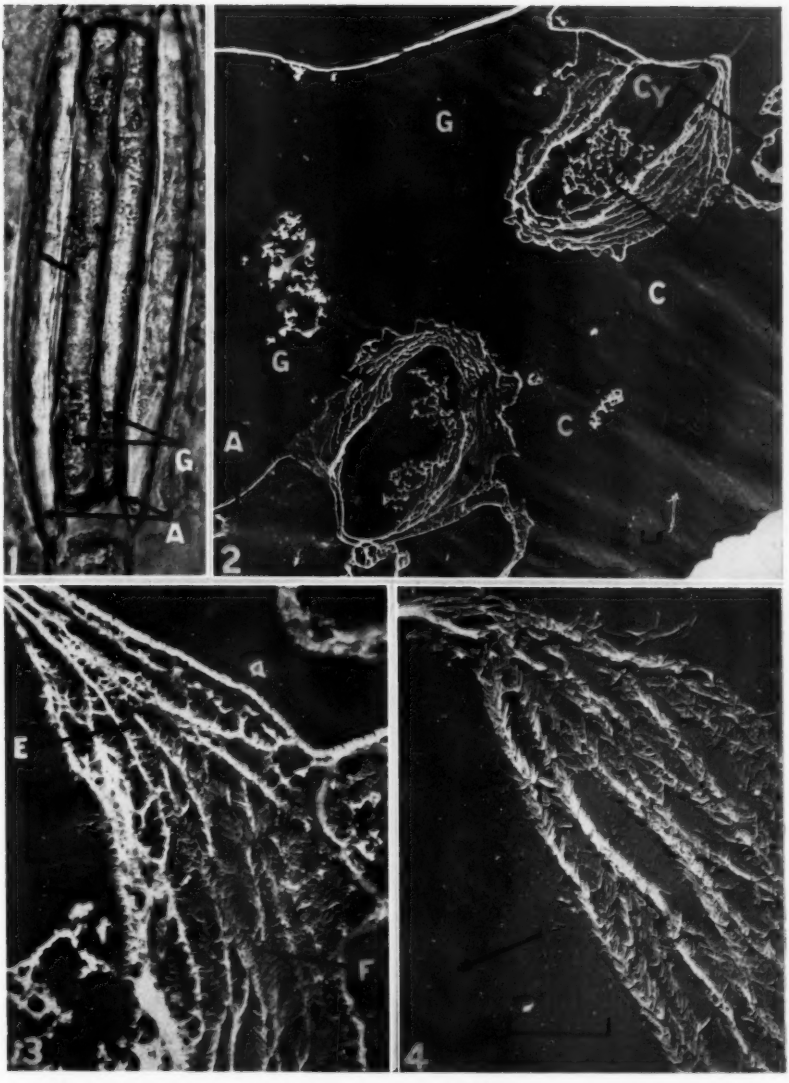


FIG. 1. Photomicrograph of a closed stoma. ×800
FIG. 2. Electron micrograph of a transverse section through a stoma. ×3000
FIG. 3. Enlarged view of area in rectangle in FIG. 2. ×12,000
FIG. 4. Electron micrograph of a transverse section through the outer wall of an epidermal cell. ×16,500
Scale lines represent 1 μ. For explanations see text.

8. ROELOFSEN, P. A. and HOUWINK, A. L. Architecture and growth of the primary cell wall in some plant hairs and in the *Phycomyces* sporangiophore. Acta Botan. Neerl. **2**, 218–225 (1953).
9. SETTERFIELD, G. and BAYLEY, S. T. Studies on the mechanism of deposition and extension of primary cell walls. Can. J. Botany, **35**, 435-444 (1957).
10. SINNOTT, E. W. and BLOCH, R. Changes in intracellular relationships during the growth and differentialion of living plant tissues. Am. J. Botany, **26**, 625-634 (1939).
11. WARDROP, A. B. The nature of surface growth in plant cells. Australian J. Botany, **4**, 193–198 (1956).

RECEIVED APRIL 29, 1957.
DIVISION OF APPLIED BIOLOGY,
NATIONAL RESEARCH LABORATORIES,
OTTAWA, CANADA.

PLANT BREEDING ABSTRACTS

A quarterly journal containing abstracts of current literature throughout the world. All publications having a direct or indirect bearing on the breeding of economic plants are mentioned. A special feature of *Plant Breeding Abstracts* is that works published in the more unfamiliar languages are abstracted extensively, including those written in other scripts such as Russian, Japanese, Chinese and Arabic. The last volume contained abstracts, in English, of articles written in 33 different languages. There are also sections devoted to notices of new books and periodicals.

PLANT BREEDING ABSTRACTS is produced and edited by the Commonwealth Bureau of Plant Breeding and Genetics, School of Agriculture, Cambridge, England, on behalf of the Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Nr. Slough, Bucks. Subscription rates are 70*s*. per volume (with subject index). Orders may be placed through booksellers or sent to: *COMMONWEALTH AGRICULTURAL BUREAUX, Central Sales Branch, Farnham House, Farnham Royal, Nr. Slough, Bucks., England.*

THE COMMONWEALTH FORESTRY BUREAU

OXFORD, ENGLAND

This branch of the Commonwealth Agricultural Bureaux, set up by the Governments of the British Commonwealth, operates as a centre for the collection and dissemination of information on forestry—not only forest botany and ecology, silviculture, protection, survey and management, but also logging and utilization, economics, administration, policy and influences.

FORESTRY ABSTRACTS: These survey current world literature on forestry, abstracting regularly from over 550 periodicals, and more than 750 serials, bulletins, etc. and countless miscellaneous publications written in some 30 languages. Each issue normally includes a review article and a news feature. Annual subscription, for the four numbers of a volume (plus a four-part annual index), is 70*s*.

GUIDE TO THE USE OF FORESTRY ABSTRACTS: An invaluable adjunct to the Abstracts, with a key to the full names and addresses of publications noticed and many other aids. Price 5*s*.

THE OXFORD SYSTEM OF DECIMAL CLASSIFICATION FOR FORESTRY: The definitive English text as approved by the International Union of Forest Research Organizations and FAO. Price 10*s*. (ordinary), 12*s*. 6*d*. (interleaved).

OCCASIONAL PUBLICATIONS AND REPRINTS OF REVIEW ARTICLES: List on request direct from *COMMONWEALTH AGRICULTURAL BUREAUX, CENTRAL SALES BRANCH, Farnham Royal, Bucks, England*, to whom all correspondence regarding publications should be addressed. All prices include postage and are payable in sterling or dollars (£1 = $2.80).

$$\frac{\partial}{\partial t}\left(\frac{\partial \psi}{\partial x}\right) + \frac{\partial}{\partial x}\left(\frac{\partial \psi}{\partial t}\right)$$

Notes to Contributors

Manuscripts

(i) ***General***

Manuscripts, in English or French, should be typewritten, double spaced, on paper 8½ × 11 in. **The original and one copy are to be submitted.** Tables and captions for the figures should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered.

Style, arrangement, spelling, and abbreviations should conform to the usage of this journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed.

Manuscripts and illustrations should be carefully checked before they are submitted. Authors will be charged for unnecessary deviations from the usual format and for changes made in the proof that are considered excessive or unnecessary.

(ii) ***Abstract***

An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

(iii) ***References***

References should be listed **alphabetically by authors' names,** numbered, and typed after the text. The form of the citations should be that used in this journal; in references to papers in periodicals, titles should be given and inclusive page numbers are required. The names of periodicals should be abbreviated in the form given in the most recent *List of Periodicals Abstracted by Chemical Abstracts*. All citations should be checked with the original articles, and each one referred to in the text by the key number.

(iv) ***Tables***

Tables should be numbered in roman numerals and each table referred to in the text. Titles should always be given but should be brief; column headings should be brief and descriptive matter in the tables confirmed to a minimum. Vertical rules should be used only when they are essential. Numerous small tables should be avoided.

Illustrations

(i) ***General***

All figures (including each figure of the plates) should be numbered consecutively from 1 up, in arabic numerals, and each figure should be referred to in the text. The author's name, title of the paper, and figure number should be written in the lower left-hand corner of the sheets on which the illustrations appear. Captions should not be written on the illustrations (see Manuscripts (i)).

(ii) ***Line drawings***

Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in blue only; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in green, yellow, or red should not be used unless it is desired to have all the co-ordinate lines show. All lines should be of sufficient thickness to reproduce well. Decimal points, periods, and stippled dots should be solid black circles large enough to be reduced if necessary. Letters and numerals should be neatly made, preferably with a stencil (**do NOT use typewriting**), and be of such size that the smallest lettering will be not less than 1 mm. high when reproduced in a cut 3 in. wide.

Many drawings are made too large; originals should not be more than 2 or 3 times the size of the desired reproduction. In large drawings or groups of drawings the ratio of height to width should conform to that of a journal page but the height should be adjusted to make allowance for the caption.

The original drawings and one set of clear copies (e.g. small photographs) are to be submitted.

(iii) ***Photographs***

Prints should be made on glossy paper, with strong contrasts. They should be trimmed so that essential features only are shown and mounted carefully, with rubber cement, on white cardboard with no space or only a **very** small space (less than 1 mm.) between them. In mounting, full use of the space available should be made (to reduce the number of cuts required) and the ratio of height to width should correspond to that of a journal page (4¼ × 7½ in.); however, allowance must be made for the captions. Photographs or groups of photographs should not be more than 2 or 3 times the size of the desired reproduction.

Photographs are to be submitted in duplicate; if they are to be reproduced in groups one set should be mounted, the duplicate set unmounted.

Reprints

A total of 50 reprints of each paper, without covers, are supplied free. Additional reprints, with or without covers, may be purchased.

Charges for reprints are based on the number of printed pages, which may be calculated approximately by multiplying by 0.6 the number of manuscript pages (doubled-spaced typewritten sheets, 8½ × 11 in.) and including the space occupied by illustrations. An additional charge is made for illustrations that appear as coated inserts. The cost per page is given on the reprint requisition which accompanies the galley.

Any reprints required in addition to those requested on the author's reprint requisition form must be ordered officially as soon as the paper has been accepted for publication.

Contents

Page

A Rapid Quantitative Test for Phytotoxicity and Its Use in the Examination of a Series of Alky-substituted Cyclohexenones—*R. A. Ludwig, Robert W. White, and C. H. Unwin* - - - - - - - - - - - - - 605

A Method for the Study of Decay Resistance in Wood under Controlled Moisture Conditions—*D. E. Etheridge* - - - - - - - - - 615

Studies on Hatching Stimulation in the Beet Nematode, *Heterodera schachtii* Schmidt—*R. D. Winslow and R. A. Ludwig* - - - - - - - - - 619

Studies of the Aging of Conidia of *Monilinia fructicola* (Wint.) Honey. I. Germination Rates and Longevity—*S. H. Z. Naqvi and H. M. Good* - - 635

Quantitative Determination of Viability of Potato Ring Rot Bacteria Following Storage, Heat, and Gas Treatments—*Lloyd T. Richardson* - - - - 647

Chromosome Numbers of Some Saskatchewan Leguminosae with Particular Reference to *Astragalus* and *Oxytropis*—*G. F. Ledingham* - - - - 657

The Lowbush Blueberry: The Vascular Anatomy of the Ovary—*Hugh P. Bell and Elspeth C. Giffin* - - - - - - - - - - - - - - 667

Definitions of Terms in Forest and Shade Tree Diseases—*D. C. Buckland, D. R. Redmond, and R. Pomerleau* - - - - - - - - - - - - - 675

Studies of the Development of *Zostera marina* L. II. Germination and Seedling Development—*A. R. A. Taylor* - - - - - - - - - 681

Studies on the Influence of Various Substances on the Germination of *Helminthosporium sativum* spores in soil—*S. H. F. Chinn and R. J. Ledingham* 697

Translocation of Radioactive Sugars in the Stems of Soybean Seedlings—*C. D. Nelson and Paul R. Gorham* - - - - - - - - - - - 703

Cytotaxonomy of *Carex* Section *Capillares*—*Åskell Löve, Doris Löve, and Marcel Raymond* - - - - - - - - - - - - - - - - - 715

Properties of a Strain of Cucumber-mosaic Virus Isolated from *Prunus* Hosts—*R. S. Willison and M. Weintraub* - - - - - - - - - - - - 763

Studies in Plant Mineral Nutrition. III. The Effects of Calcium Concentration in Culture Solutions upon the Absorption of Iron and Manganese by Dwarf Kidney Bean—*C. D. Taper and W. Leach* - - - - - - - - 773

Chromosome Numbers of Canadian Weeds. I—*Gerald A. Mulligan* - - - 779

Notes:

Fine Structure of Guard-cell Walls in *Avena* Coleoptile—*George Setterfield* - 791

Hi's Hi's